

Antioxidant and Antimicrobial Effects of Green-Synthesized Nanoparticles Using Several Plants Wastewater

Bazı Bitki Atık Suları Kullanılarak Yeşil Sentezlenmiş Nanopartiküllerin Antioksidan ve Antimikrobiyal Etkileri

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Abstract

This study was aimed to green synthesise of iron, zinc and copper nanoparticles using wastewater from steam distillation of *Helichrysum arenarium* plant and needles of *Pinus sylvestris* and *Picea orientalis*. SEM, EDX, and UV-Vis were used for the characterization of green-synthesized nanoparticles. *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* strains were used to assess antimicrobial activity. Antioxidant activity was tested using DPPH free radical scavenging method. Green-synthesized nanoparticles from steam distillation wastewater of *P. orientalis* were found to have sizes ranging from 322-366 nm for FeNPs, 650-950 nm for ZnNPs, and 460-560 nm for CuNPs. EDX analysis showed that FeNPs contained 5.68% iron, ZnNPs contained 0.43% zinc, and CuNPs contained 0.82% copper. Two distinct peaks between 280 and 300 nm were observed in the UV-Vis spectra of the Fe, Zn and Cu nanoparticles. In this study, no antimicrobial activity was observed for FeNPs, ZnNPs, and CuNPs. However, the scavenging percentages of free radicals were found to be 36.48%, 62.40% and 57.88% respectively. As a result, FeNPs, ZnNPs, or CuNPs were produced using green synthesis methods with plant wastewater from steam distillation, and the nanoparticles exhibited effective antioxidant properties. The results demonstrate that plant wastewater produced by steam distillation has significant potential for nanoparticle production, as well as plant extracts. This study is important as it shows that plant wastewater, classified as waste and generated by the steam distillation process, can contribute to the production of nanoparticles with antioxidant effects.

Öz

Bu çalışmada, *Helichrysum arenarium* bitkisinin, *Pinus sylvestris* ve *Picea orientalis* ibrelerinin buhar distilasyonu sonucu oluşan atık suları kullanılarak demir, çinko ve bakır nanopartikülleri yeşil sentezlenmesi amaçlanmıştır. Yeşil sentezlenmiş nanopartiküller, SEM görüntüleme, EDX bileşim analizi ve UV-Vis spektroskopisi yardımıyla karakterize edilmiştir. Antimikrobiyal aktivite *Pseudomonas aeruginosa*, *Staphylococcus aureus* ve *Candida albicans* üzerinde test edilmiştir. Antioksidan aktivite, serbest radikal süpürme yöntemiyle DPPH (2,2-difenil-1-pikrilhidrazil) üzerinde test edilmiştir. *P. orientalis* buhar distilasyonu atık suyundan yeşil sentezlenmiş nanopartiküller ve boyutları sırasıyla FeNPs 322-366 nm, ZnNPs 650-950 nm ve CuNPs 460-560 nm arasında belirlenmiştir. Ayrı ayrı yapılan EDX analizi sonuçlarında FeNPs %5.68 demir, ZnNPs %0.43 çinko ve CuNPs %0.82 bakır içerdikleri belirlenmiştir. FeNPs, ZnNPs ve CuNPs ait UV-Vis spektrumlarında 280-300 nm arasında iki farklı pik değerleri gözlenmiştir. SEM, EDX ve UV-Vis analiz sonuçlarıyla Fe, Zn ve Cu nanopartiküllerinin başarılı bir şekilde sentezlendiği doğrulanmıştır. Çalışmada, Fe, Zn ve Cu nanopartiküllerinin antimikrobiyal aktiviteleri gözlenmemiştir. Ancak Fe, Zn ve Cu nanopartiküllerinin serbest radikal süpürme yüzdeleri sırasıyla %36.48, %62.40 ve %57.88 olarak belirlenmiştir. Sonuç olarak buhar distilasyonu sonucu oluşan bitki atık suları kullanılarak yeşil sentez yöntemiyle FeNPs, ZnNPs ve CuNPs üretilmiş ve bu nanopartiküllerin etkili antioksidan özellik gösterdikleri belirlenmiştir. Bu sonuçlar, buhar distilasyonu sonucu oluşan bitki atık sularının da bitki ekstraktları gibi nanopartikül üretiminde önemli bir potansiyele sahip olduklarını ortaya koymuştur. Bu durum kaplama ve indirgeyici bileşiklere sahip olduklarını düşündürmektedir. Bu çalışma, atık su olarak sınıflandırılan ve buhar distilasyonu işlemi sonucunda oluşan bitki atık sularının antioksidan etkiye sahip nanopartiküllerin üretimini sağlaması açısından büyük önem taşımaktadır.

INTRODUCTION

Nanoparticles (NPs) are a wide-ranging group of materials comprising particulate matter measuring at least 100 nm in size. Depending on their general shape, they can be in zero-, one-, two- or three-dimensional. NPs are generally categorised based on their size, morphology and chemical properties. Due to their size, morphology and distribution, nanoparticles exhibit advanced properties and are widespread usage in different scientific areas. Biological materials including plants and microorganisms are less expensive and more environmentally friendly (Wu et al. 2008, Khan et al. 2019).

There are two basic methods for synthesising nanoparticles. Those are top-down and bottom-up synthesis methods. In the bottom-up synthesis method, chemical synthesis or green synthesis methods involving redox reactions are utilized. In the top-down synthesis method, nanoparticles are prepared from bulk materials. A variety of analytical methods are employed to characterize NPs, including X-ray diffraction analysis (XRD), UV-Vis, fourier transform infrared spectroscopy (FTIR), selected area electron diffraction (SAED), differential scanning calorimetry (DSC), zeta potential, dynamic light scattering (DLS), energy dispersive spectroscopy (EDS), transmission electron microscopy (TEM), thermogravimetric analysis (TGA) and scanning electron microscopy (SEM) (Wang et al. 2025). The adsorption of hazardous chemicals onto surface of the synthesized NPs during chemical synthesis limits NPs usage for therapeutic and medical purposes.

The limitations of existing chemical methods have led to the development of a simpler, more energy-efficient, cost-effective and environmentally friendly method of producing NPs with natural agents such as bacteria, plant extracts, fungi, microorganisms and yeast. These environmentally friendly synthesis approaches are becoming increasingly popular with the aim of eliminating polluting, toxic and hazardous agents.

Furthermore, NPs synthesized by these methods are non-toxic, highly environmentally friendly, and more dimensionally accurate than those produced using traditional methods. The term “green technology” has recently gained significant importance because NPs have ushered in a new era of safe nanotechnology through green synthesis or biological synthesis. This synthesis is significant because the number of published articles on this topic has steadily increased in recent years. The production of NPs from metal ions is based on the remarkable ability of phytochemicals present in biological extracts, including metabolites, proteins, sugars, flavanols and polyphenols, to produce NPs (Rani et al. 2023).

Currently, green synthesis techniques for nanoparticles are being proposed over traditional synthesis techniques because they offer several advantages in terms of cost, energy consumption, non-toxicity, and safety. Green synthesis is defined as the application of biologically friendly elements such as bacteria, plants, and fungi for the nanoparticle synthesis. Green synthesis of iron nanoparticles is an environmentally friendly, reliable, and sustainable protocol for synthesizing nanoparticles. Numerous plants exist in nature and are of pharmacological importance (Kumar et al. 2023a). Green nanoparticles are synthesized from plants using extracts from leaves, fruits, bark, seeds, etc (Şahin et al. 2024). The plant extract acts as both a reducing and capping agent in the synthesis of nanoparticles (Hano and Abbasi 2021).

Flavonoids, polyphenols, and other bioactive phytochemicals play an important role in the green synthesis of metal ion nanoparticles. The conversion of metal ions into nanoparticles is facilitated by these naturally occurring compounds, which act as reducing agents. Nanomaterials interact more effectively with microbial cells due to their high surface-to-volume ratio and nanoscale size. This interaction can interrupt the structures of cell membrane, inhibit vital enzymatic activities, and cause cell death. Due to their strong antimicrobial activities, NPs such as copper and zinc oxide are attracting significant interest (Semwal et al. 2025).

Researchers also use plant extracts to synthesize iron nanoparticles because of their polyphenol content, which converts iron ions into zero-valent iron (Mohamed et al. 2023). It has been noted that, when optimal conditions are provided, metal nanoparticles produced by green synthesis methods are of much higher quality than those produced by traditional methods (Saxena et al. 2025). Plant extracts are commonly used in the production of green nanoparticles (Rathod et al. 2024, Roopa et al. 2025). Plants are used to synthesize nanoparticles because they have multiple biochemical properties, are widely available, inexpensive, and easy to mass-produce (Safa and Koohestani 2024).

In recent years, green-synthesized nanoparticles have attracted significant interest due to their potential applications in fields such as nanomedicine (Beg et al. 2017), antibacterial (Gur 2022, Elsaffany et al. 2025), antifungal (Wasilewska et al. 2023), antimicrobial (Alex et al. 2024), antioxidant (Elsaffany et al. 2025), anticancer (Alharbi and Alsubhi 2022, Alex et al. 2024), anti-inflammatory (Pandiyan et al. 2022, Elsaffany et al. 2025), protection against DNA damage (Gur 2022), drug nanocarrier and gene delivery (Hamimed et al. 2022). *Helichrysum* species are frequently used in various medical remedies (Taghavizadeh Yazdi et al. 2020). *Helichrysum arenarium* extract has been used in the green synthesis of silver (Ozdemir et al. 2023, Kahraman 2024, Mphahlele et al. 2024, Şahin et al. 2024, Yilmaz et al. 2024), copper (Şahin et al. 2024), zinc (Meydan and Seçkin 2021) and gold (Ertas Onmaz et al. 2022) nanoparticles. *Pinus sylvestris* extract has been used in the green synthesis of silver nanoparticles (Macovei et al. 2023, Rahmatian et al. 2023).

Approximately 13% of the flora of Türkiye is found in the province of Artvin. Artvin is one of the top provinces in Türkiye in terms of plant species and endemic species diversity. There are 1.268 plant species within the borders of Artvin province, 119 of which are endemic (URL-1). The steam distillation (SD) is the most widely used method to extract essential oils from plants (Stratakos and Koidis 2016). The main principle of this method is to distil the mixture of compounds from plants at a temperature lower than their boiling point (Fongang Fotsing Yannick et al. 2021, Ghenabzia et al. 2023). The process of steam distillation, used to obtain essential oils, produces large amounts of wastewater and solid plant residues. Disposing of this waste materials in the environment can cause various problems.

These waste materials are biological pollutants that are rich in biologically active compounds. Scientists are researching the usability of these waste materials in different fields. However, the chemical properties and detailed profiles of these waste materials are still unknown. Only volatile compounds are obtained through the distillation process, while non-volatile compounds pass into the waste fraction. These non-volatile compounds are also valuable biological materials (Gateva et al. 2022). Wastewater contains non-volatile compounds after the steam distillation process. The potential applications of biologically active compounds in various fields have been investigated. Plant-based agents are well known for use in nanoparticle synthesis due to their environmentally friendly nature. However, plant extracts can serve as both a coating and a reducing agent in nanoparticle synthesis. Converting these non-volatile compounds into value-added biological materials is therefore important.

In this study, the aim was to synthesize iron, zinc and copper nanoparticles from *H. arenarium* plant and the needles of *P. sylvestris* and *P. orientalis* using steam distillation wastewater, and then to investigate the potential antioxidant and antimicrobial activity of these nanoparticles.

MATERIALS AND METHODS

Materials

Nutrient broth (Merck 105443), nutrient agar (Merck 105450), DPPH (Sigma-Aldrich D9132), ethanol (Supelco ACS reagent, ≥99.9% (GC), 1.00983), FeCl₂ (Sigma-Aldrich 8.03945), CuCl₂·2H₂O (Supelco ACS reagent 1.02733), and ZnCl₂ (Sigma-Aldrich, puriss. 14422) were used.

Methods

Collection of Steam Distillation Wastewater

The Medicinal and Aromatic Plants Application and Research Center at Artvin Çoruh University frequently extracts essential oils from medicinal aromatic plants via steam distillation. Wastewater from these plants was collected following steam distillation. Steam distillation wastewater of *Helichrysum arenarium* (L.) Moench plant, *Pinus sylvestris* Thunb. needles and *Picea orientalis* (L.) Peterm needles—a liquid aqueous residue used in the present study were generated because of water steam distillation done in Medicinal and Aromatic Plants Application and Research Center at Artvin Çoruh University in Artvin in Türkiye. Since plant wastewaters were used, no identification or characterization of the plant sources was performed. The wastewater from the steam distillation process was collected separately and stored in darkness at room temperature.

Green Synthesis of Nanoparticles

Green synthesis of NPs was performed following Lakshminarayanan et al. (2021) using *H. arenarium* plant material and the needles of *P. sylvestris* and *P. orientalis* steam distillation wastewater, with minor modifications. Solutions of FeCl₂, CuCl₂.2H₂O, and ZnCl₂ salts were prepared at a concentration of 0.1 M in 50 mL of distilled water and boiled for 120 min at 120°C. Then 50 mL of plant wastewater was added dropwise to 50 mL of the metal salt solutions. After the reaction was complete, the supernatant was discarded via centrifugation for 5 min at 6000 rpm. The pellets were then rinsed with distilled water, followed by ethanol. The synthesized nanoparticles were characterized by SEM, EDX and UV-Vis analyses.

Free Radical Scavenging Activity

Antioxidant activity of green synthesized nanoparticles was tested via 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity with minor modifications (Canbolat et al. 2021, Yu et al. 2002). Firstly, the nanoparticles with 25 and 50 µg/mL concentration were added onto 0.1 mM DPPH free radical solution and incubated in the dark at room temperature for 30 minutes. After incubation, the absorbance values were measured at 517 nm using a microplate reader. The results were calculated as percent inhibition. The percent inhibition value was calculated using the formula in Equation 1.

$$\text{Inhibition\%} = \frac{\text{AbsControl} - \text{AbsSample}}{\text{AbsControl}} \times 100 \quad \text{Equation 1}$$

Antimicrobial Activity

The antimicrobial (antibacterial and antifungal) activities of green synthesis nanoparticles were determined using the well diffusion method with minor modifications (Magaldi et al. 2004, Valgas et al. 2007, Hussein-Al-Ali et al. 2014). For this purpose, Gram (-) negative (*Pseudomonas aeruginosa* ATCC 27853), Gram (+) positive (*Staphylococcus aureus* ATCC 25923) and yeast (*Candida albicans* ATCC 10231) were cultured overnight in NB medium at 37°C. After incubation, the microorganisms were thoroughly spread on the surface of agar plates and 50 µL of nanoparticle solutions at 1 mg/mL concentration were added into wells (approximately 8 mm in diameter) on the agar plates. Inhibition zones that occurred after the plates incubation were measured. Sterile distilled water was used as a control. The results obtained were compared with standard antimicrobial discs. Antimicrobial activity was tested in triplicate.

RESULTS AND DISCUSSION

Green Synthesis of Nanoparticles

Green synthesis offers several advantages over traditional chemical synthesis methods. It is cheaper, reduces pollution and improves environmental and public health and safety (Ying et al. 2022). The green synthesis of nanomaterials relies on bacteria, yeasts, fungi, algal species and certain plants (Huston et al. 2021). The most obvious indicators in nanoparticle synthesis are color change and precipitate formation (Aigbe and Osibote 2024, Ibrahim et al. 2024). Numerous studies have reported that the solution changes color and precipitation is observed during the green synthesis of nanoparticles (Lakshminarayanan et al. 2021, Sepasgozar et al. 2021, Can and Kizilbey 2024, Ibrahim et al. 2024, Manimehala et al. 2025). In our study we also observed color change and precipitation during the synthesis of nanoparticles using *H. arenarium* plant, needles of *P. sylvestris* and *P. orientalis* steam distillation wastewater. Iron, zinc, and copper nanoparticles have been successfully synthesized using *P. sylvestris* and *P. orientalis* steam distillation wastewater, respectively. However, sufficient amounts of nanoparticles were not obtained from *P. sylvestris* steam distillation wastewater. Therefore, further studies have been conducted on nanoparticles which synthesized with *P. orientalis* steam distillation wastewater.

Characterization

SEM-EDX Analysis

SEM analyses of NPs were analysed at a magnification of 20.00 Kx. Figure 1a showed that the FeNPs were in clusters and have an irregular spherical morphology with a size range of 322–366 nm. The presence of FeNPs was confirmed by the peaks at around 0.7 and 6.4 keV, which correspond to the binding energies of Fe in the EDX spectra (Rahman et al. 2017). In Figure 1b, the EDX spectrum reveals the content of carbon (57.19%), oxygen (37.13%), and iron (5.68%) in the FeNPs.

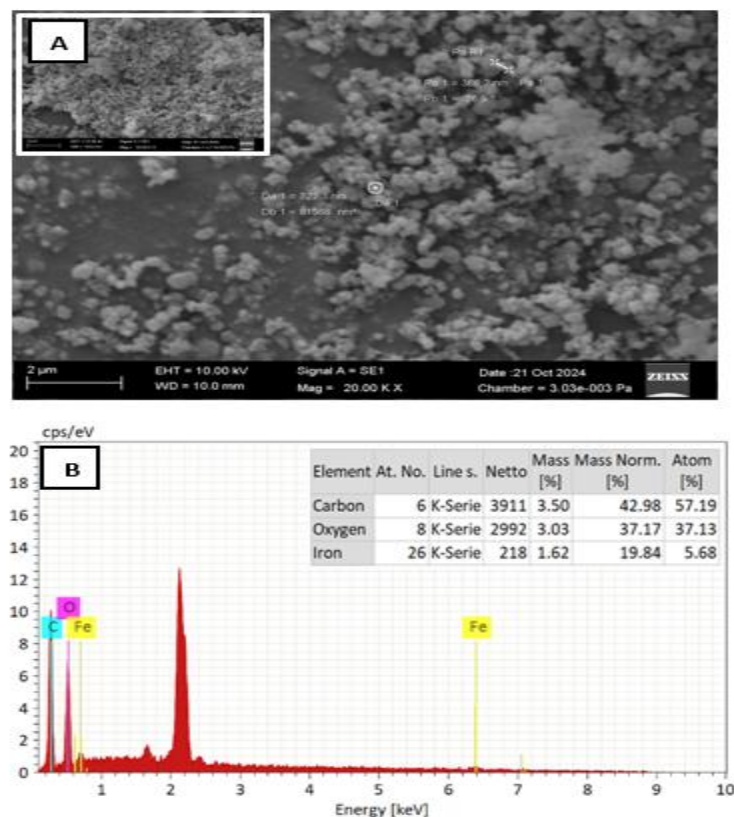


Figure 1. SEM micrographs (A) and EDX analysis (B) of synthesized FeNPs using *P. orientalis* steam distillation wastewater

In Figure 2a, ZnNPs appeared as nanorods and clusters with sizes ranging from 650 to 950 nm. In Figure 2b, the presence of ZnNPs was confirmed by the peaks seen at around 1 keV, 8.6 keV and 9.6 keV in the EDX spectra, corresponding emission peaks of Zn (Naiel et al. 2022). The EDX spectrum of the ZnNPs showed the presence of carbon (63.92%), oxygen (34.06%) and zinc (0.43%).

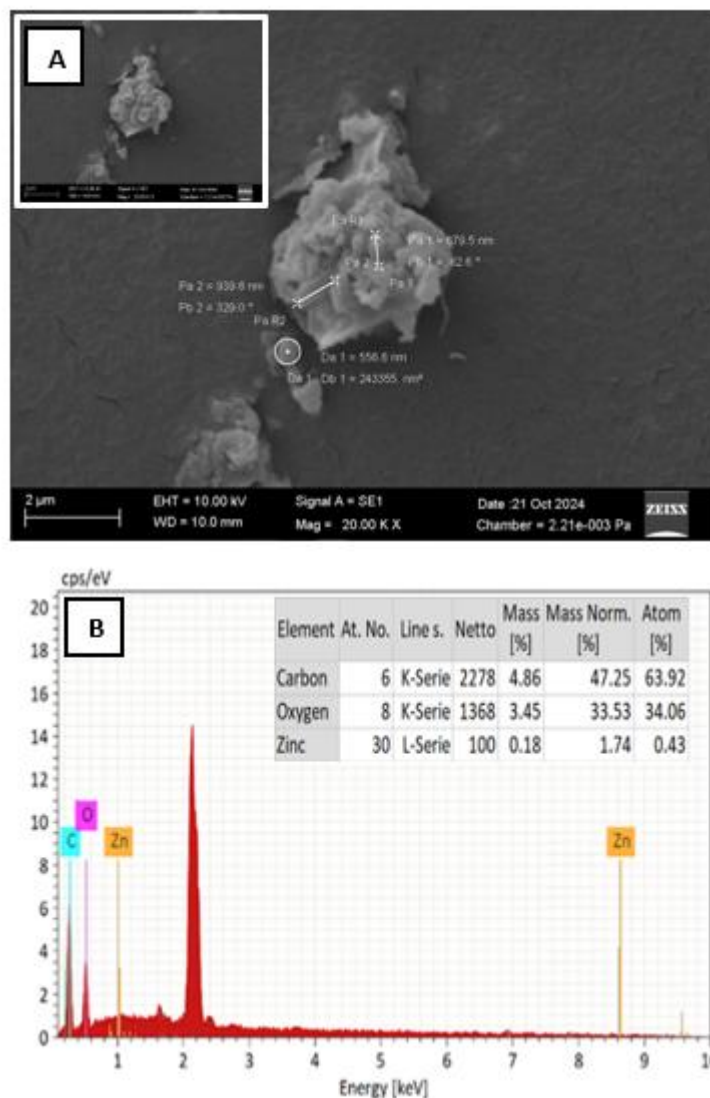


Figure 2. SEM micrographs (A) and EDX analysis (B) of synthesized ZnNPs using *P. orientalis* steam distillation wastewater

CuNPs were observed to have spherical shape with sizes ranging from 460 to 560 nm, as shown in Figure 3a. It has been stated that synthesized CuNPs can exhibit a wide size distribution, approximately 20 nm to 300 nm (Semisch et al. 2014, Khaldari et al. 2021). In Figure 3b, the EDX spectrum of the synthesized CuNPs indicates the presence of copper at specific peaks at ~1 keV, 8 keV and 9 keV, confirming successful synthesis of nanoparticles (Nkosi et al. 2025). The EDX spectrum of the CuNPs showed contents of carbon (62.24%), oxygen (36.93%), and copper (0.82%).

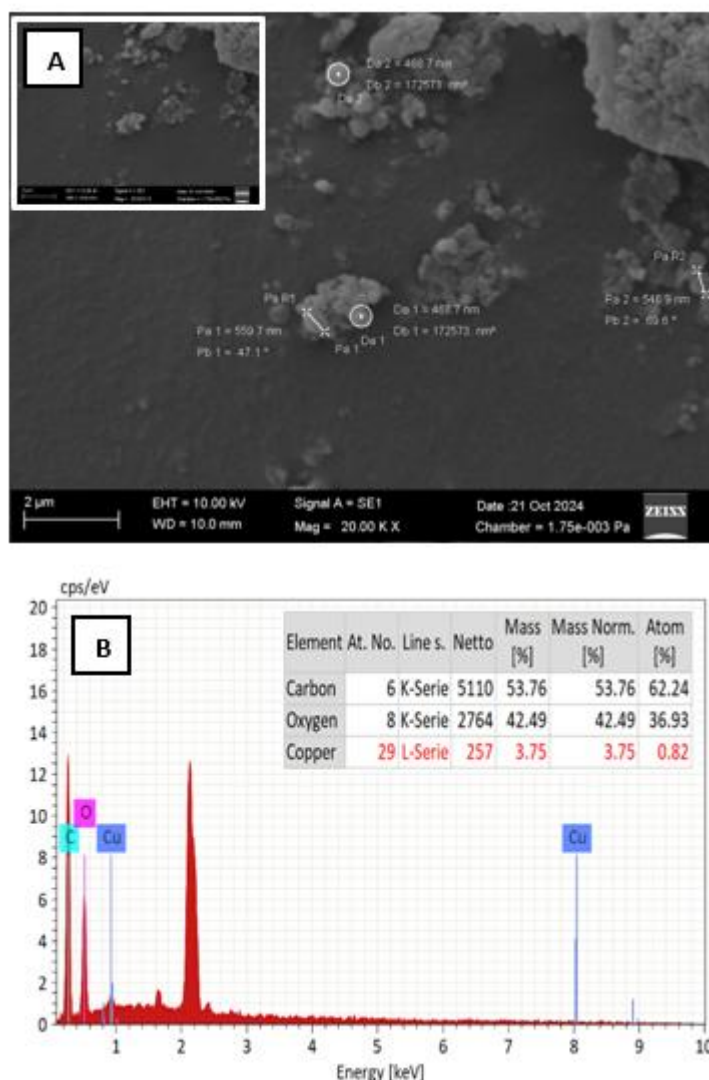


Figure 3. SEM micrographs (A) and EDX analysis (B) of synthesized CuNPs using *P. orientalis* steam distillation wastewater

UV-Vis Analysis

In our study, scanning wavelength measurements were performed from 200 to 800 nm of synthesized FeNPs, ZnNPs and CuNPs using *P. orientalis* steam distillation wastewater. Figure 4 reveals that UV-Vis analyses for FeNPs (Figure 4a), ZnNPs (Figure 4b) and CuNPs (Figure 4c) showed two peak values appearing at 280-290 nm. However, the peak points of these two peaks exhibit differences for each metal. Our results were consistent with the data and findings obtained in other studies.

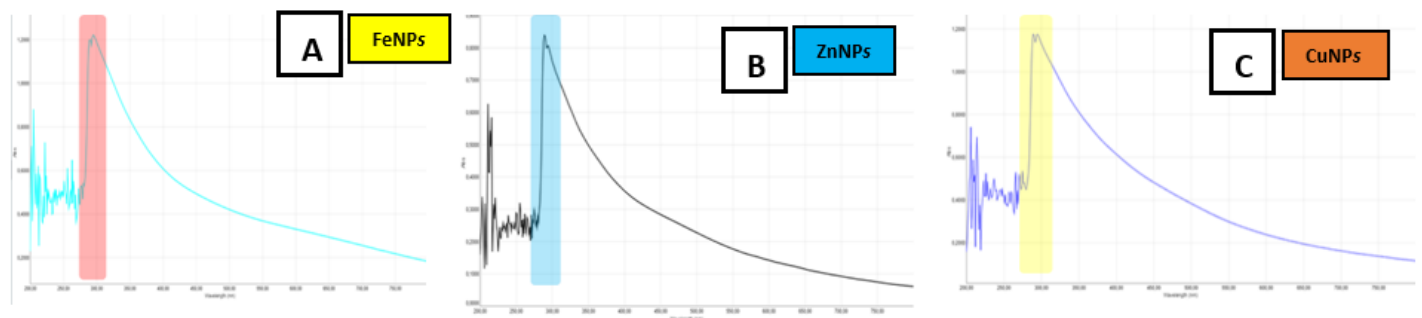


Figure 4. UV-Vis analysis results of synthesized FeNPs (A), ZnNPs (B) and CuNPs (C) nanoparticles using *P. orientalis* steam distillation wastewater

Free Radical Scavenging Activity

In our study, antioxidant activity was evaluated based on the free radical scavenging activity on DPPH. The antioxidant activity of green-synthesized nanoparticles was tested at concentrations of 25 and 50 $\mu\text{g/mL}$. An increase in antioxidant activity was observed with increasing concentration. At 25 $\mu\text{g/mL}$, CuNPs showed 37.75%, FeNPs 21.26%, and ZnNPs 38.83% free radical scavenging activity, while at 50 $\mu\text{g/mL}$, CuNPs showed 57.88%, FeNPs 36.48%, and ZnNPs 62.40% free radical scavenging activity. The highest antioxidant activity was observed with $\text{Zn} > \text{Cu} > \text{Fe}$ nanoparticles. The results of the antioxidant activity assays were shown in Figure 5. In our study, it was observed that the antioxidant activities of nanoparticles increased with increasing concentrations. Imon et al. (2024) observed inhibition rates of approximately 26–29% at a concentration of 1000 $\mu\text{g/mL}$. Raj et al. (2025) reported that significant free radical scavenging activity of 78.4% was observed at a concentration of 1000 μg . The percentage of DPPH inhibition increased depending on the concentration (Benshiga et al. 2024, Imon et al. 2024, Islam et al. 2024, Palani et al. 2024, Shah et al. 2024, Khajuria et al. 2025, Raj et al. 2025). These antioxidant activity results in the literature are consistent with our current study.

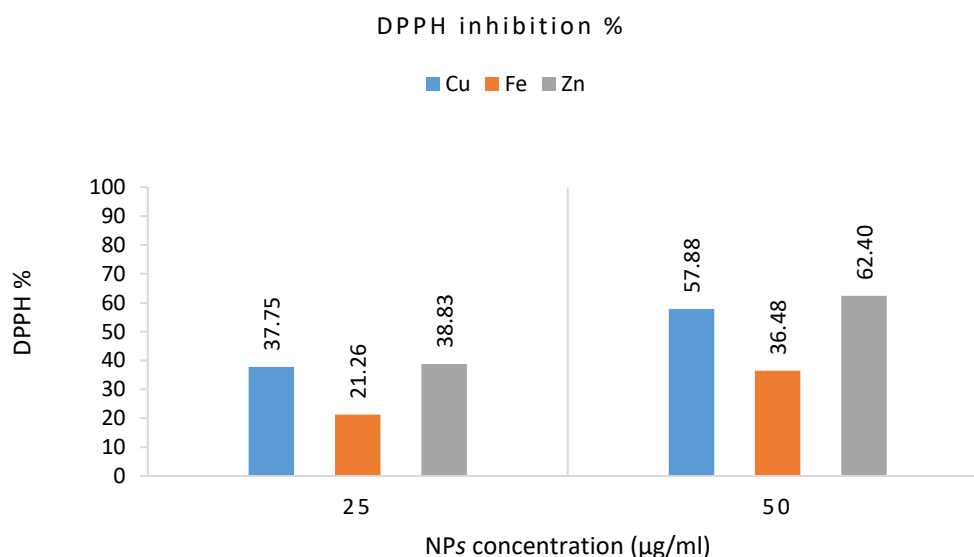


Figure 5. Results of nanoparticle- and concentration-dependent antioxidant activity of synthesized FeNPs, ZnNPs and CuNPs nanoparticles using *P. orientalis* steam distillation wastewater

Antimicrobial Activity

Steam distillation makes it easier to separate compounds that vaporise below their normal boiling points. Volatile components absorb heat from the steam and are carried away with the water vapour (Shrivastava 2023). In green synthesis nanoparticle studies, it has been noted that plant extracts are used for the synthesis (Abdullah et al. 2020, Ashrafi-Saiedlou et al. 2025) which are rich in phytochemical compounds (Dubale et al. 2023, Kumar et al. 2023b, Uy et al. 2025). Various plant volatiles have been reported to have the best broad-spectrum antibacterial activity (Abers et al. 2021, Ahmad et al. 2021). Some literature reports the observation of inhibition zones (Gunalan et al. 2012, Manimehala et al. 2025, Roy et al. 2025, Sharma and Kaur 2025) even at 1000 µg/mL concentration of NPs (Ashwini et al. 2021). In our study green synthesized Fe, Zn and Cu nanoparticles using *P. orientalis* steam distillation wastewater showed no inhibition zones at 1000 µg/mL. The absence of antimicrobial effects in our study is thought to be due to the fact that the bioactive compounds with antimicrobial activity present in the plant being carried away with the steam or separated in the form of volatile oils as a result of steam distillation. The results of the antimicrobial activity tests are shown in Table 1. The inhibition zone diameter (millimetre) is the mean value.

Table 1. Antimicrobial results of green synthesized nanoparticles using *P. orientalis* steam distillation wastewater

Samples	Gram (-)	Gram (+)	Yeast
	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>C. albicans</i>
CuNPs	8.0	8.0	8.0
FeNPs	8.0	8.0	8.0
ZnNPs	8.0	8.0	8.0
dH ₂ O	8.0	8.0	8.0

Concentration: 1000 µg/mL, Well diameter: 8 mm

DISCUSSION

As a result of steam distillation, two different products are obtained: essential oils and hydrosol. However, two different types of waste are also produced: plant waste and distillation wastewater. This study investigated the green synthesis of nanoparticles using wastewater obtained from the steam distillation of various plants. Therefore, iron, zinc, and copper nanoparticles were synthesized using steam distillation wastewater from the *H. arenarium* plant and the needles of *P. sylvestris* and *P. orientalis*. No significant color change or precipitation was observed at the end of the synthesis in the Fe, Zn and Cu solutions, which were prepared using wastewater from the steam distillation of the *H. arenarium* plant.

In contrast, color change and precipitation were observed in Fe, Zn and Cu solutions which used wastewater from steam distillation of *P. sylvestris* and *P. orientalis* needles. However, FeNPs, ZnNPs and CuNPs were not significantly produced using steam distillation wastewater of *P. sylvestris* needles. Therefore, further studies were conducted on nanoparticles which were synthesized using the steam distillation wastewater from *P. orientalis* needles. SEM was employed to analyses the synthesized nanoparticles via size distribution and surface morphology. SEM analyses of NPs were analysed at a magnification of 20.00 Kx. Sezen et al. (2023) reported an average particle size of 1000 nm for the green synthesis nanoparticles. They also found that the nanorods consisted of irregular particles. Additionally, it has been reported that these nanorods adhere to each other.

Mengesha et al. (2024) have pointed out that SEM morphologies reveal the unique structural characteristics of each nanoparticle type. SEM images showed that CuNPs exhibited an irregular spherical particle distribution and a varied morphology. It has also been noted that the difference in shape may be due to the different precursor materials used in the green synthesis method. The presence of FeNPs was confirmed by the peaks at around 0.7 and 6.4 keV which are

linked to the binding energies of Fe in the EDX spectra (Rahman et al. 2017). The presence of ZnNPs was confirmed by the peaks seen at around 1 keV, 8.6 keV and 9.6 keV in the EDX spectra, which correspond to the emission peaks of Zn.

It has also been stated that carbon emission peaks at ~0.3 keV and oxygen emission peaks at ~0.5 keV may originate from the use of plant biomass in green synthesis (Naiel et al. 2022). The EDX spectrum of CuNPs indicated the presence of copper at specific peaks at ~1 keV, 8 keV and 9 keV, confirming the successful synthesis of the nanoparticles (Nkosi et al. 2025). UV-Vis Spectrometer reveals the nanoparticles characteristic formation during the color change based on absorption spectra (Lakshminarayanan et al. 2021). In the literature, it has been stated that the peaks formed between 230 and 290 nm as a result of UV-Vis analysis confirm the presence of iron oxide nanoparticles (Rauf et al. 2025).

As a result of green synthesis, it has been reported that the iron oxide nanoparticles UV-Vis spectrum exhibits a sharp peak at 290 nm which occurred due to the surface plasmon resonance (Yekeen et al. 2025). It was also stated that the synthesized iron oxide nanoparticles were confirmed by UV-Vis spectroscopy with the peak at 295 nm (Rauf et al. 2025). It has been stated that the UV-Vis spectrum of green synthesized ZnO NPs shows a strong absorption peak at approximately 370 nm (Naiel et al. 2022). UV-Vis spectrum analysis showed that the absorbance observed between 250 and 300 nm indicated the formation of CuO NPs (Sepasgozar et al. 2021). However, UV-Vis analysis results with different peaks have also been reported for ZnNPs and CuNPs (Ashwini et al. 2021, Naiel et al. 2022, Tsilo et al. 2023, Nkosi et al. 2025). Although it has been stated in the literature that UV-Vis is used for the characterization of nanoparticles, it has been noted that peaks can occur at similar wavelengths. The literatures indicate that green-synthesized nanoparticles exhibit effective antioxidant properties (Ashrafi-Saiedlou et al. 2025, Nughwal et al. 2025).

In antioxidant activity studies, the degree of color loss depends on the concentration of the antioxidant (Palani et al. 2024). This indicates that DPPH is reduced by donating a hydrogen atom and exhibits high scavenging activity (Wu et al. 2020). The most important feature of green synthesized NPs is the electrostatic interaction between positively charged metal ions in NPs and negatively charged bioactive compounds in plant extracts. This interaction greatly increases antioxidant activity (Tiwari et al. 2024). Plant-based essential oils and extracts contain a wide variety of bioactive and volatile compounds in varying concentrations. Therefore, investigating the bioactivity of compounds classified as waste may lead to the development of novel natural antimicrobial agents, thereby maximizing the plant resources utilization. Although the literature has focused heavily on the use of essential oils, the research on distillation residues as bioactive components has been less studied (Mitropoulou et al. 2025).

Reyes-Jurado et al. (2015) reported that 32–35 components are obtained as a result of steam distillation, while 22 compounds are observed as a result of organic solvent extraction. This suggests that the number of components in the essential oils obtained through steam distillation has increased, while the number of substances passing into the wastewater has decreased. Extracts from different plant species used in the green synthesis of NPs act as stabilizing and reducing agents. In the current study, the synthesized nanoparticles exhibited no antimicrobial effect against the test microorganisms, but they exhibited effective antioxidant activity on DPPH. It is believed that this is the first study in which nanoparticles have been synthesized with the steam distillation wastewater of plants.

CONCLUSION

This study showed that the wastewater generated by the steam distillation can also be used in the green synthesis of metal NPs including Cu, Fe and Zn. Green synthesized NPs were confirmed by SEM imaging, EDX composition analysis, and UV-vis spectroscopy. The NPs sizes were measured to be between 300 and 950 nm. UV-Vis spectrums showed two peak values appearing between 280 and 300 nm. The NPs demonstrated improved antioxidant activity via scavenging DPPH free radicals. These results highlight the potential of green-synthesized NPs as an alternative to traditionally

synthesized NPs, which produce toxic effects, in addressing the adverse effects caused by free radicals. The study demonstrates the practical applications of green-synthesized NPs in sustainable and effective therapeutic formulations using water considered waste as a result of steam distillation, providing opportunities for further research and commercialization. This study is significant in evaluating the wastewater produced by steam distillation of plants and implementing zero-waste applications.

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REFERENCES

- Abdullah JAA, Eddine LS, Abderrhmane B, Alonso-González M, Guerrero A, Romero A (2020) Green synthesis and characterization of iron oxide nanoparticles by *Pheonix dactylifera* leaf extract and evaluation of their antioxidant activity. *Sustainable Chemistry and Pharmacy*, 17:100280. <https://doi.org/10.1016/j.scp.2020.100280>
- Abers M, Schroeder S, Goelz L, Sulser A, St Rose T, Puchalski K, Langland J (2021) Antimicrobial activity of the volatile substances from essential oils. *BMC Complementary Medicine and Therapies*, 21(1):124. <https://doi.org/10.1186/s12906-021-03285-3>
- Ahmad A, Elisha IL, van Vuuren S, Viljoen A (2021) Volatile phenolics: A comprehensive review of the anti-infective properties of an important class of essential oil constituents. *Phytochemistry*, 190:112864. <https://doi.org/10.1016/j.phytochem.2021.112864>
- Aigbe UO, Osibote OA (2024) Green synthesis of metal oxide nanoparticles, and their various applications. *Journal of Hazardous Materials Advances*, 13:100401. <https://doi.org/10.1016/j.hazadv.2024.100401>
- Alex AM, Subburaman S, Chauhan S, Ahuja V, Abdi G, Tarighat MA (2024) Green synthesis of silver nanoparticle prepared with *Ocimum* species and assessment of anticancer potential. *Scientific Reports*, 14(1):11707. <https://doi.org/10.1038/s41598-024-61946-y>
- Alharbi NS, Alsubhi NS (2022) Green synthesis and anticancer activity of silver nanoparticles prepared using fruit extract of *Azadirachta indica*. *Journal of Radiation Research and Applied Sciences*, 15(3):335-345. <https://doi.org/10.1016/j.jrras.2022.08.009>
- Ashrafi-Saiedlou S, Rasouli-Sadaghiani M, Fattahi M (2025) Green synthesis of iron oxide nanoparticles using *Thymus migricus* for multifunctional applications in antioxidant, antimicrobial, photocatalytic, and seed priming processes. *Heliyon*, 11(5): e42933. <https://doi.org/10.1016/j.heliyon.2025.e42933>
- Ashwini J, Aswathy TR, Rahul AB, Thara GM, Nair AS (2021) Synthesis and characterization of zinc oxide nanoparticles using *Acacia caesia* bark extract and its photocatalytic and antimicrobial activities. *Catalysts*, 11(12):1507. <https://doi.org/10.3390/catal11121507>
- Beg M, Maji A, Mandal AK, Das S, Aktara MN, Jha PK, Hossain M (2017) Green synthesis of silver nanoparticles using *Pongamia pinnata* seed: characterization, antibacterial property, and spectroscopic investigation of interaction with human serum albumin. *Journal of Molecular Recognition*, 30(1):e2565. <https://doi.org/10.1002/jmr.2565>
- Benshiga E, Sivalingam AM, Alex A, Neha B, Brahma N (2024) In vitro antioxidant activity of green-synthesized zinc oxide (ZnO) nanoparticles utilizing extracts from *Allium sativum*. *Cureus*, 16(2): e55184. <https://doi.org/10.7759/cureus.55184>
- Can A, Kızılbey K (2024) Green synthesis of ZnO nanoparticles via *Ganoderma Lucidum* extract: structural and functional analysis in polymer composites. *Gels*, 10(9):576. <https://doi.org/10.3390/gels10090576>
- Canbolat D, Turan İ, Küpeli YE, Kılınc S, Piliç S (2021) Artvin Şavşat yöresi propolisinin farklı sıcaklıklardaki PBS'li ekstraktlarının antioksidan özelliklerinin ve eritrosit hemoliz inhibisyonu üzerine etkisinin araştırılması. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, 24(3):464-472. <https://doi.org/10.18016/ksutarimdog.vi.765838>
- Dubale S, Kebebe D, Zeynudin A, Abdissa N, Suleman S (2023) Phytochemical screening and antimicrobial activity evaluation of selected medicinal plants in Ethiopia. *Journal of Experimental Pharmacology*, 15:51-62. <https://doi.org/10.2147/JEP.S379805>
- Elsaffany AH, Abdelaziz AE, Zahra AA, Mekky AE (2025) Green synthesis of silver nanoparticles using cocoon extract of *Bombyx mori* L.: therapeutic potential in antibacterial, antioxidant, anti-inflammatory, and anti-tumor applications. *BMC Biotechnology*, 25(1):38. <https://doi.org/10.1186/s12896-025-00971-9>
- Ertas Onmaz N, Demirezen Yılmaz D, Imre K, Morar A, Gungor C, Yılmaz S, Gundog DA, Dishan A, Herman V, Gungor G (2022) Green synthesis of gold nanoflowers using *Rosmarinus officinalis* and *Helichrysum italicum* extracts: comparative studies of their antimicrobial and antibiofilm activities. *Antibiotics*, 11(11):1466. <https://doi.org/10.3390/antibiotics11111466>
- Fongang Fotsing Yannick S, Bankeu Kezet Jean J, Gaber El-Saber B, Iftikhar A, Lenta Ndjaku B (2021) Extraction of Bioactive Compounds from Medicinal Plants and Herbs. In: Hany A. El-Shemy (Ed) *Natural Medicinal Plants*, 1st Edn. IntechOpen, London, pp:147-185. <https://doi.org/10.5772/intechopen.98602>
- Gateva S, Jovtchev G, Angelova T, Dobрева A, Mileva M (2022) The anti-genotoxic activity of wastewaters produced after water-steam distillation of Bulgarian *Rosa damascena* Mill. and *Rosa alba* L. essential oils. *Life*, 12(3):455. <https://doi.org/10.3390/life12030455>
- Ghenabzia I, Hemmami H, Amor IB, Zeghoud S, Seghir BB, Hammoudi R (2023) Different methods of extraction of bioactive compounds and their effect on biological activity: a review. *International Journal of Secondary Metabolite*, 10(4):469-494. <https://doi.org/10.21448/ijsm.1225936>
- Gunalan S, Sivaraj R, Rajendran V (2012) Green synthesized ZnO nanoparticles against bacterial and fungal pathogens. *Progress in Natural Science: Materials International*, 22(6):693-700. <https://doi.org/10.1016/j.pnsc.2012.11.015>
- Gur T (2022) Green synthesis, characterizations of silver nanoparticles using sumac (*Rhus coriaria* L.) plant extract and their antimicrobial and DNA damage protective effects. *Frontiers in Chemistry*, 10:968280. <https://doi.org/10.3389/fchem.2022.968280>
- Hamimed S, Jabberi M, Chatti A (2022) Nanotechnology in drug and gene delivery. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 395(7):769-787. <https://doi.org/10.1007/s00210-022-02245-z>

- Hano C, Abbasi BH (2021) Plant-based green synthesis of nanoparticles: production, characterization and applications. *Biomolecules*, 12(1):31. <https://doi.org/10.3390/biom12010031>
- Hussein-Al-Ali SH, El Zowalaty ME, Kura AU, Geilich B, Fakurazi S, Webster TJ, Hussein MZ (2014) Antimicrobial and controlled release studies of a novel nystatin conjugated iron oxide nanocomposite. *Biomed Research International*, 2014(1):651831. <https://doi.org/10.1155/2014/651831>
- Huston M, DeBella M, DiBella M, Gupta A (2021) Green synthesis of nanomaterials. *Nanomaterials*, 11(8):2130. <https://doi.org/10.3390/nano11082130>
- Ibrahim NH, Taha GM, Hagaggi NSA, Moghazy MA (2024) Green synthesis of silver nanoparticles and its environmental sensor ability to some heavy metals. *BMC Chemistry*, 18(1):7. <https://doi.org/10.1186/s13065-023-01105-y>
- Imon AI, Toma SN, Sohag SM, Islam J, Islam M, Sohag SU, Mahmud I, Shahria N, Dutta S (2024) Evaluation of anthelmintic and antioxidant efficacy of green-synthesized copper nanoparticles derived from *Erioglossum rubiginosum* leaf and seed aqueous extracts. *European Journal of Medicinal Chemistry Reports*, 12:100181. <https://doi.org/10.1016/j.ejmcr.2024.100181>
- Islam F, Islam S, Miah AS, Huq AKO, Saha AK, Mou ZJ, Mondol MH, Bhuiyan MNI (2024) Green synthesis of zinc oxide nano particles using *Allium cepa* L. waste peel extracts and its antioxidant and antibacterial activities. *Heliyon*, 10(3):e25430. <https://doi.org/10.1016/j.heliyon.2024.e25430>
- Kahraman HT (2024) Synthesis of silver nanoparticles using *Alchemilla vulgaris* and *Helichrysum arenarium* for methylene blue and 4-nitrophenol degradation and antibacterial applications. *Biomass Conversion and Biorefinery*, 14(12):13479-13490. <https://doi.org/10.1007/s13399-024-05314-w>
- Khajuria AK, Kandwal A, Sharma R, Bachheti RK, Worku LA, Bachheti A (2025) In vitro antioxidant and antibacterial activities of biogenic synthesized zinc oxide nanoparticles using leaf extract of *Mallotus philippinensis* Mull. Arg. *Scientific Reports*, 15(1):6541. <https://doi.org/10.1038/s41598-025-85264-z>
- Khaldari I, Naghavi MR, Motamedi E (2021) Synthesis of green and pure copper oxide nanoparticles using two plant resources via solid-state route and their phytotoxicity assessment. *RSC Advances*, 11(6):3346-3353. <https://doi.org/10.1039/D0RA09924D>
- Khan I, Saeed K, Khan I (2019) Nanoparticles: properties, applications and toxicities. *Arabian Journal of Chemistry*, 12(7):908-931. <https://doi.org/10.1016/j.arabjch.2017.05.011>
- Kumar A, Nirmal P, Kumar M, Jose A, Tomer V, Oz E, Proestos C, Zeng M, Elobeid T, Sneha K, Oz F (2023b) Major phytochemicals: recent advances in health benefits and extraction method. *Molecules*, 28(2):887. <https://doi.org/10.3390/molecules28020887>
- Kumar V, Kaushik NK, Tiwari SK, Singh D, Singh B (2023a) Green synthesis of iron nanoparticles: sources and multifarious biotechnological applications. *International Journal of Biological Macromolecules*, 253(4):127017. <https://doi.org/10.1016/j.ijbiomac.2023.127017>
- Lakshminarayanan S, Shereen MF, Niraimathi KL, Brindha P, Arumugam A (2021) One-pot green synthesis of iron oxide nanoparticles from *Bauhinia tomentosa*: characterization and application towards synthesis of 1, 3 diolein. *Scientific Reports*, 11(1):8643. <https://doi.org/10.1038/s41598-021-87960-y>
- Macovei I, Luca SV, Skalicka-Woźniak K, Horhoge CE, Rimbu CM, Sacarescu L, Vochita G, Cherghel D, Ivanescu BL, Panainte AD, Nechita C, Corciova A, Miron A (2023) Silver nanoparticles synthesized from *Abies alba* and *Pinus sylvestris* bark extracts: characterization, antioxidant, cytotoxic, and antibacterial effects. *Antioxidants*, 12(4):797. <https://doi.org/10.3390/antiox12040797>
- Magaldi S, Mata-Essayag S, De Capriles CH, Pérez C, Colella M, Olaizola C, Ontiveros Y (2004) Well diffusion for antifungal susceptibility testing. *International Journal of Infectious Diseases*, 8(1):39-45. <https://doi.org/10.1016/j.ijid.2003.03.002>
- Manimehala U, Asha S, Tomy M, Anu MA, Sumitha MS, Kumar P, Xavier TS, Binoy J (2025) Green synthesis of copper oxide nanoparticles from *Camellia sinensis* extract: effects of calcination temperatures on antimicrobial activity. *Biochemical and Biophysical Research Communications*, 769:151963. <https://doi.org/10.1016/j.bbrc.2025.151963>
- Mengesha SM, Abebe GM, Habtemariam TH (2024) Biosynthesis of CuO nanoparticle using leaf extracts of *Ocimum lamiifolium* Hochst. ex Benth and *Withania somnifera* (L) Dunal for antibacterial activity. *Scientific Reports*, 14(1):1-12. <https://doi.org/10.1038/s41598-024-75296-2>
- Meydan İ, Seçkin H (2021) Green synthesis, characterization, antimicrobial and antioxidant activities of zinc oxide nanoparticles using *Helichrysum arenarium* extract. *International Journal of Agriculture Environment and Food Sciences*, 5(1):33-41. <https://doi.org/10.31015/iaefs.2021.1.5>
- Mitropoulou G, Karapantzou I, Tsimogiannis D, Oreopoulou V, Lazăr V, Kourkoutas Y (2025) Inhibitory effects of essential oils and extracts of the water-steam distillation residues from greek herbs on adherent biofilm formation by common pathogens. *Applied Sciences*, 15(6):3385. <https://doi.org/10.3390/app15063385>
- Mohamed A, Atta RR, Kotp AA, Abo El-Ela FI, Abd El-Raheem H, Farghali A, Alkhalifah DHM, Hozzein WN, Mahmoud R (2023) Green synthesis and characterization of iron oxide nanoparticles for the removal of heavy metals (Cd²⁺ and Ni²⁺) from aqueous solutions with antimicrobial Investigation. *Scientific Reports*, 13(1):7227. <https://doi.org/10.1038/s41598-023-31704-7>
- Mphahlele LL, Sekoai PT, Gbadeyan OJ, Ramdas V, Ramchuran S, Chunilall V, Mkhize M (2024) Optimization of setpoint conditions for enhanced biofabrication of silver nanoparticles using *Helichrysum crispum* extracts. *Nanomaterials*, 14(23):1916. <https://doi.org/10.3390/nano14231916>
- Naiei B, Fawzy M, Halmy MWA, Mahmoud AED (2022) Green synthesis of zinc oxide nanoparticles using Sea Lavender (*Limonium pruinosum* L. Chaz.) extract: characterization, evaluation of anti-skin cancer, antimicrobial and antioxidant potentials. *Scientific Reports*, 12(1):20370. <https://doi.org/10.1038/s41598-022-24805-2>
- Nkosi NC, Basson AK, Ntombela ZG, Dlamini NG, Pullabhotla RV (2025) Green synthesis of copper nanoparticles using a bioflocculant from *Proteus mirabilis* AB 932526.1 for wastewater treatment and antimicrobial applications. *Applied Nano*, 6(1):5. <https://doi.org/10.3390/applnano6010005>
- Nughwal A, Bharti R, Thakur A, Verma M, Sharma R, Pandey A (2025) Green synthesis of iron oxide nanoparticles from Mexican prickly poppy (*Argemone mexicana*): assessing antioxidant activity for potential therapeutic use. *RSC Advances*, 15(13):10287-10297. <https://doi.org/10.1039/D4RA07232D>
- Ozdemir C, Gencer M, Coksu I, Ozbek T, Derman S (2023) A new strategy to achieve high antimicrobial activity: green synthesised silver nanoparticle formulations with *Galium aparine* and *Helichrysum arenarium*. *Archives of Industrial Hygiene and Toxicology*, 74(2):90. <https://doi.org/10.2478/aiht-2023-74-3684>
- Palani M, Kalaiselvan S, Mark JAM, Chandran K, Ekambaram V (2024) Green synthesis of CuO nanoparticles: a promising role of antioxidant and antimicrobial activity by using *Tribulus terrestris* L. *Aspects of Molecular Medicine*, 4:100049. <https://doi.org/10.1016/j.amolm.2024.100049>

- Pandiyan I, Sri SD, Indiran MA, Rathinavelu PK, Prabakar J, Rajeshkumar S (2022) Antioxidant, anti-inflammatory activity of *Thymus vulgaris*-mediated selenium nanoparticles: an in vitro study. *Journal of Conservative Dentistry and Endodontics*, 25(3):241-245. https://doi.org/10.4103/JCD.JCD_369_21
- Rahman SSU, Qureshi MT, Sultana K, Rehman W, Khan MY, Asif MH, Farooq M, Sultana N (2017) Single step growth of iron oxide nanoparticles and their use as glucose biosensor. *Results in Physics*, 7:4451-4456. <https://doi.org/10.1016/j.rinp.2017.11.001>
- Rahmatian N, Abbasi S, Yarak MT, Abbasi N (2023) *Echinophora platyloba* extract-mediated green synthesis of silver nanoparticles: fine-tuning the size towards enhanced catalytic and antibacterial properties. *Journal of Molecular Liquids*, 391:123327. <https://doi.org/10.1016/j.molliq.2023.123327>
- Raj LA, Pavithra R, Namasivayam SKR (2025) Green route synthesis of highly stable zinc oxide nanoparticles using root extract of *Andrographis paniculata* and evaluation of their potential activities. *Plant Nano Biology*, 12:100162. <https://doi.org/10.1016/j.plana.2025.100162>
- Rani N, Singh P, Kumar S, Kumar P, Bhankar V, Kumar K (2023) Plant-mediated synthesis of nanoparticles and their applications: a review. *Materials Research Bulletin*, 163:112233. <https://doi.org/10.1016/j.materresbull.2023.112233>
- Rathod S, Preetam S, Pandey C, Bera SP (2024) Exploring synthesis and applications of green nanoparticles and the role of nanotechnology in wastewater treatment. *Biotechnology Reports*, 41: e00830. <https://doi.org/10.1016/j.btre.2024.e00830>
- Rauf A, Ahmad Z, Ajaj R, Zhang H, Ibrahim M, Muhammad N, Al-Awthan YS, Bahattab OS, Ullah I (2025) Green synthesis an eco-friendly route for the synthesis of iron oxide nanoparticles using aqueous extract of *Thevetia peruviana* and their biological activities. *Scientific Reports*, 15(1):18316. <https://doi.org/10.1038/s41598-025-02387-z>
- Reyes-Jurado F, Franco-Vega A, Ramírez-Corona N, Palou E, López-Malo A (2015) Essential oils: antimicrobial activities, extraction methods, and their modeling. *Food Engineering Reviews*, 7:275-297. <https://doi.org/10.1007/s12393-014-9099-2>
- Roopa M, Thirumala S, Kallimani S, Manohara B, Basavarajaiah S (2025) Green synthesis of nanoparticles for enhanced wastewater treatment and other applications: a review and future perspectives. *Next Materials*, 8:100664. <https://doi.org/10.1016/j.nxmte.2025.100664>
- Roy DRS, Aman R, Chand U, Kushawaha PK (2025) Green synthesis of zinc nanoparticles using leaf extract of *Clitoria ternatea* and evaluation of its antimicrobial and anti-oxidant activity. *The Microbe*, 7:100290. <https://doi.org/10.1016/j.microb.2025.100290>
- Safa MAT, Koohestani H (2024) Green synthesis of silver nanoparticles with green tea extract from silver recycling of radiographic films. *Results in Engineering*, 21:101808. <https://doi.org/10.1016/j.rineng.2024.101808>
- Saxena R, Kotnala S, Bhatt S, Uniyal M, Rawat B, Negi P, Riyal MK (2025) A review on green synthesis of nanoparticles toward sustainable environment. *Sustainable Chemistry for Climate Action*, 6:100071. <https://doi.org/10.1016/j.scca.2025.100071>
- Semisch A, Ohle J, Witt B, Hartwig A (2014) Cytotoxicity and genotoxicity of nano- and microparticulate copper oxide: role of solubility and intracellular bioavailability. *Particle and Fibre Toxicology*, 11(1):10. <https://doi.org/10.1186/1743-8977-11-10>
- Semwal A, Gautam K, Bhatt M, Yadav R, Barthwal S, Singh H (2025) Green synthesis and application of metal nanoparticles (MNPs): a sustainable strategy for protecting wood from fungal and termite decay. *Next Materials*, 9:100962. <https://doi.org/10.1016/j.nxmte.2025.100962>
- Sepasgozar SME, Mohseni S, Feizyadeh B, Morsali A (2021) Green synthesis of zinc oxide and copper oxide nanoparticles using *Achillea nobilis* extract and evaluating their antioxidant and antibacterial properties. *Bulletin of Materials Science*, 44(2):129. <https://doi.org/10.1007/s12034-021-02419-0>
- Sezen S, Ertugrul MS, Balpınar Ö, Bayram C, Özkaraca M, Okkay IF, Hacımuftuoğlu A, Güllüce M (2023) Assessment of antimicrobial activity and In Vitro wound healing potential of ZnO nanoparticles synthesized with *Capparis spinosa* extract. *Environmental Science and Pollution Research*, 30(55):117609-117623. <https://doi.org/10.1007/s11356-023-30417-8>
- Shah SS, Turakhia BP, Purohit N, Kapadiya KM, Sahoo CR, Prajapati A (2024) Facile green synthesis of iron oxide nanoparticles and their impact on cytotoxicity, antioxidative properties and bactericidal activity. *Iranian Biomedical Journal*, 28(2-3):71. <https://doi.org/10.61186/ibi.4061>
- Sharma A, Kaur A (2025) *Catharanthus roseus* leaf-based green synthesis of silver oxide nanoparticles: characterization, phytochemicals screening and antimicrobial activity. *The Microbe*, 8:100469. <https://doi.org/10.1016/j.microb.2025.100469>
- Shrivastava A (2023) Steam Distillation: Principle and Applications for the Extraction of Essential Oils from Plants. In: Arunachalam K, Yang X, Puthanpura Sasidharan S (Eds) *Bioprospecting of Tropical Medicinal Plants*. Springer, Cham. pp:893-903. https://doi.org/10.1007/978-3-031-28780-0_36
- Stratakos AC, Koidis A (2016) Methods for extracting essential oils. *Essential Oils in Food Preservation, Flavor and Safety*, 31-38. <https://doi.org/10.1016/B978-0-12-416641-7.00004-3>
- Şahin M, Arslan Y, Soyucok A (2024) *Helichrysum arenarium*-mediated facile green synthesis, antibacterial, catalytic activity and hydrogen evolution of metallic (Ag, Cu) and bimetallic (Ag/Cu) nanoparticles. *Materials Chemistry and Physics*, 314:128853. <https://doi.org/10.1016/j.matchemphys.2023.128853>
- Taghavizadeh Yazdi ME, Amiri MS, Akbari S, Sharifalhosseini M, Nourbakhsh F, Mashreghi M, Yousefi E, Abbasi MR, Modarres M, Es-haghi A (2020) Green synthesis of silver nanoparticles using *Helichrysum graveolens* for biomedical applications and wastewater treatment. *BioNanoScience*, 10(4):1121-1127. <https://doi.org/10.1007/s12668-020-00794-2>
- Tiwari AK, Jha S, Tripathi SK, Shukla R, Awasthi RR, Bhardwaj AK, Singh AK, Dikshit A (2024) Spectroscopic investigations of green synthesized zinc oxide nanoparticles (ZnO NPs): antioxidant and antibacterial activity. *Discover Applied Sciences*, 6(8):399. <https://doi.org/10.1007/s42452-024-06049-z>
- Tsilo PH, Basson AK, Ntombela ZG, Dlamini NG, Pullabhotla RV (2023) Biosynthesis and characterization of copper nanoparticles using a bioflocculant produced by a yeast *Pichia kudriavzevii* isolated from Kombucha tea SCOBY. *Applied Nano*, 4(3):226-239. <https://doi.org/10.3390/applnano4030013>
- URL-1 Geographical Situation. <http://www.artvin.gov.tr/cografi-durum>, Accessed: 06.11.2025.
- Uy NP, Ku J, Doo-Hee L, Nam SJ, Lee S (2025) Comparative assessment of phytochemical content and antioxidant activities in different parts of *Pyrus ussuriensis* cultivars. *Horticulturae*, 11(2):184. <https://doi.org/10.3390/horticulturae11020184>
- Valgas C, de Souza SM, Smânia EFA, Smânia Jr A (2007) Screening methods to determine antibacterial activity of natural products. *Brazilian Journal of Microbiology*, 38(2):369-380. <https://doi.org/10.1590/S1517-83822007000200034>
- Wang Z, Wei J, Cai Z, Dai Y, Wu Q (2025) A review of edible mushroom-mediated green synthesis of nanoparticles and their emerging biomedical potentials. *Inorganic Chemistry Communications*, 181(1):115129. <https://doi.org/10.1016/j.inoche.2025.115129>

- Wasilewska A, Klekotka U, Zambrzycka M, Zambrowski G, Świącicka I, Kalska-Szostko B (2023) Physico-chemical properties and antimicrobial activity of silver nanoparticles fabricated by green synthesis. *Food Chemistry*, 400:133960. <https://doi.org/10.1016/j.foodchem.2022.133960>
- Wu W, He Q, Jiang C (2008) Magnetic iron oxide nanoparticles: synthesis and surface functionalization strategies. *Nanoscale Research Letters*, 3(11):397. <https://doi.org/10.1007/s11671-008-9174-9>
- Wu S, Rajeshkumar S, Madasamy M, Mahendran V (2020) Green synthesis of copper nanoparticles using *Cissus vitiginea* and its antioxidant and antibacterial activity against urinary tract infection pathogens. *Artificial Cells, Nanomedicine, and Biotechnology*, 48(1):1153-1158. <https://doi.org/10.1080/21691401.2020.1817053>
- Yekeen MO, Ibrahim M, Wachira J, Pramanik S (2025) Green synthesis and characterization of iron oxide nanoparticles using *Egeria densa* plant extract. *Applied Biosciences*, 4(2):27. <https://doi.org/10.3390/applbiosci4020027>
- Yilmaz MT, İspirli H, Taylan O, Balubaid M, Dertli E (2024) Facile biomimetic synthesis of AgNPs using aqueous extract of *Helichrysum arenarium*: characterization and antimicrobial activity. *Inorganic and Nano-Metal Chemistry*, 54(7):627-638. <https://doi.org/10.1080/24701556.2022.2081204>
- Ying S, Guan Z, Ofoegbu PC, Clubb, P, Rico C, He F, Hong J (2022) Green synthesis of nanoparticles: current developments and limitations. *Environmental Technology & Innovation*, 26:102336. <https://doi.org/10.1016/j.eti.2022.102336>
- Yu L, Haley S, Perret J, Harris M, Wilson J, Qian M (2002) Free radical scavenging properties of wheat extracts. *Journal of Agricultural and Food Chemistry*, 50(6):1619-1624. <https://doi.org/10.1021/jf010964p>