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Investigation of Antibacterial and Antifungal Efficacy of Zinc and Silver Nanoparticles Synthesized from *Nasturtium officinale*

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ABSTRACT

Nanoparticles are nano-sized materials that can be widely used in fields such as medicine, pharmacology, and industry. The use of natural and easily available materials in nanoparticle synthesis is preferable for economic reasons. Plants are extremely suitable for the synthesis of nanoparticles due to their wide availability and the large number of components they contain with various properties. For this purpose, silver nanoparticles and zinc nanoparticles (AgNPs and ZnNPs), two different nanoparticles were synthesized from an edible plant, watercress (*Nasturtium officinale*). Scanning electron microscopy, scanning electron microscopy-energy dispersive X-ray, UV-VIS spectroscopy, X-ray diffraction (XRD), and fourier transform infrared spectrophotometer (FTIR) analyses of these nanoparticles were performed. In addition, the antimicrobial effects of these synthesized nanoparticles were determined using the disk diffusion method. The nanoparticles obtained from *Nasturtium officinale* were effective on Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*), Gram-positive bacteria (*Staphylococcus aureus, Streptococcus pyogenes*), and fungi (*Candida albicans*). In particular, AgNPs with broad-spectrum antimicrobial activity were obtained from the watercress. While ZnNPs showed inhibition effects of 49% on *K. pneumoniae*, 51% on *S. aureus*, and 62% on *C. albicans*, AgNPs showed inhibition effects of 93% on *P. aeruginosa*, 87% on *S. aureus*, 81% on *E. coli*, 80% on *C. albicans*, 72% on *K. pneumoniae*, and 56% on *S. pyogenes*. The results show that *Nasturtium officinale* can be used effectively in the production of new biotechnological products, particularly ones with antimicrobial properties.

Keywords: Antimicrobial activity, Biotechnological products, Green synthesis, Medicinal plants, Metallic nanoparticles

1. Introduction

Plants and their secondary metabolites have important applications in medicine and pharmacology due to their antimicrobial, antioxidant, and anti-inflammatory properties (Punetha et al. 2022; Özkan et al. 2023). *Nasturtium officinale* is a plant belonging to the Brassicaceae family and is a perennial dicotyledonous herb that typically lives in water (Barker 2009). *Nasturtium officinale* is considered to be a traditional medicinal plant due to its many beneficial components such as vitamins, bioactive components, and glucosinolates (Gonçalves et al. 2009; Pourhassan-Moghaddam et al. 2014). In addition, *N. officinale* can be used in the treatment of both rhinitis and urinary tract irritations and is also suitable for use in cosmetic products (Klimek-Szczykutowicz et al. 2018). For these reasons, *N. officinale* is a good option for green nanoparticle synthesis.

Nanoparticles are materials that can find uses from medicine to food. Nanotechnology is an interdisciplinary field of research (Natarajan et al. 2010; Ibrahim 2015). Nanoparticle synthesis is typically carried out by various chemical methods that require the use of expensive or harmful solvents (Saini et al. 2013). For this reason, efforts are being made to find new environmentally friendly methods in the synthesis of metal nanoparticles (Hubenthal 2011; Kouvaris et al. 2012). Silver nanoparticles are relatively more important than other metal nanoparticles due to their chemical stability, conductivity, and antibacterial, antifungal, antiviral, and anti-inflammatory properties (Adebayo-Tayo et al. 2022; Khan et al. 2022) and can be used in the food industry, superconducting materials, and cosmetics (Singhal et al. 2011; Ahmed et al. 2016; Khan et al. 2022). In addition, silver nanoparticles are used in textile and medical devices, water filtration, and the diagnosis and treatment of some diseases (Geraldes et al. 2015; Khan et al. 2022). ZnO and Zn nanoparticles are added to paints, food supplements, batteries, as well as materials such as plastics, ceramics, cement, and rubber due to their electrical and thermal conductivity, stability, and antimicrobial effects (Moezzi et al. 2012; Sturikova et al. 2018). In particular, environmentally friendly green methods created using plants are preferable due to their low costs. For this purpose, studies have been carried out to synthesize more reliable agents by creating green nanoparticles with antimicrobial properties (Yang et al. 2021). In recent years, particularly, the green synthesis of nanoparticles has attracted widespread attention because the material used is both natural and cheap (Moodley et al. 2020). The chemical properties of the synthesized nanoparticles vary depending on the amount, size, and shape. It is known that many of these properties are controlled by the dispersion of nanoparticles (Heilmann 2003; Slistan-Grijalva et al. 2005; Saini et al. 2013). Due to these different properties, nanoparticles can be used both in the diagnosis of diseases and as an antimicrobial agent (Kouvaris et al. 2012; Parveen et al. 2012) in the medical field, as well as in many fields, such as electronics (Shiju & Guliants 2009; Phillips et al. 2011), the chemical industry (Kim et al. 2010; Kouvaris et al. 2012).

Today, the resistance of bacteria to antibiotics used in the treatment of diseases is an important public health problem (Moodley et al. 2020). The inability to control infections due to antibiotic resistance may cause an increase in patient deaths as well as an increase in healthcare costs (Moodley et al. 2020). The antimicrobial properties of plants and nanoparticles derived from plants have been recently investigated (Ozdek et al. 2020; Subramanyam et al. 2021). For this reason, in this study, AgNPs and ZnNPs green nanoparticles from *Nasturtium officinale* were synthesized and their antimicrobial effects were investigated.

2. Material and Methods

2.1. Materials

Watercress collected from its natural environment in the province of Kayseri was used. The authentication of the plant was performed by Prof. Dr. Hasan AKAN at Harran University and stored Harran University with the herbarium number 6363.

Tested microorganisms; *P. aeruginosa* ATCC 9027, *E. coli* ATCC 11229, *S. aureus* ATCC 25923, *C. albicans* ATCC 10231, *K. pneumoniae* ATCC 13883, strains were purchased from Microbiologics. *S. pyogenes* (ATCC 19615) was obtained from the Ankara Refik Saydam Public Health Center Presidency.

Chemicals; Nutrient agar medium, broth medium (Condalab brand), and erythromycin (15 µg) were purchased from Bioanalyse. $AgNO_3$ and $ZnSO_47H_2O$ were purchased from Sigma-Aldrich (Germany).

2.2. Methods

One hundred g of watercress (*Nasturtium officinale*), which was collected from its natural environment and dried, was taken and mixed with 1000 mL of water in a magnetic stirrer heated at 60 °C for four hours and then passed through filter paper. Prepared 100 mL of 1 mM $AgNO_3$ and 0.1 M 200 mL of $ZnSO_4$ solutions were mixed with 400 mL each of *Nasturtium officinale* extract and incubated for 24 hours. The formation of ZnNPs and AgNPs was confirmed by measuring the absorbance at 400-800 nm (Pugazhendhi et al. 2018). Then, after centrifugation at 5000 rpm for 30 minutes, the solid phase was collected in a tube and dried in an oven at 60 °C. SEM, SEM EDX, XRD, and Fourier transform infrared spectrophotometer (FTIR) analyzes of the synthesized nanoparticles were performed. In addition, solutions of AgNPs and ZnNPs nanoparticles were prepared with distilled water at concentrations of 20 mg/mL. Afterwards, its antimicrobial activity was investigated on Gram-positive bacteria (*S. aureus, S. pyogenes*), Gram-negative bacteria (*E. coli, P. aeruginosa, K. pneumoniae*), and fungi (*C. albicans*).

2.3. UV-VIS spectroscopy, SEM, SEM EDX, XRD, and FTIR analysis

Spectroscopic analyzes were performed using a BIOCHROM S70DUAL UV-VIS spectrophotometer between 300-800 nm. SEM and SEM EDX analyzes were performed using a JEOL JSM 6510 scanning electron microscope (SEM) at the 500V-30kV operating range, x5-x300,000 magnification range. An XRD analysis was performed with a Parallel Beam Scan, and Bragg-Brentano Scan, using a D/teX Ultra detector at 40kV. Descriptive analyzes of the bonds in the molecular structure of nanoparticles were performed using a Perkin Elmer Spectrum 100 FT-IR spectrophotometer.

2.4. Antimicrobial activity

The disk diffusion method was used to determine the antimicrobial activity (NCCLS 1997). Microorganisms from fresh culture were incubated at 37 °C to 0.5 Mcfarland turbidity. The absorbance at 625 nm was adjusted to be 0.08-0.10 in the spectrophotometer. Bacteria and fungi were grown in the prepared broth and then transferred to the solid medium. Then, solutions of AgNPs and ZnNPs at 20 mg/mL concentrations were absorbed into sterile discs. Inhibition zone diameters were measured for *E. coli*, *P. aeruginosa*, *S. aureus*, *S. pyogenes,* and *K. pneumoniae* bacteria after 24 hours of incubation at 37 °C, and after 48 hours of incubation at 30 °C for *C. albicans*. The same procedure was repeated for the positive control erythromycin (15 µg) and the negative control distilled water.

2.5. Statistical analysis

All measurements were made in triplicate and the results are given as mean values ± standard deviations. The antimicrobial activity of AgNPs and ZnNPs nanoparticles was investigated by One-Way ANOVA statistical analysis.. The results showed significant antimicrobial activity. The significance between groups was $p<0.05$.

3. Results and Discussion

3.1. UV-VIS analysis of AgNPs synthesized from Nasturtium officinale

The UV-VIS spectroscopy plot of AgNPs is given in Figure 1.

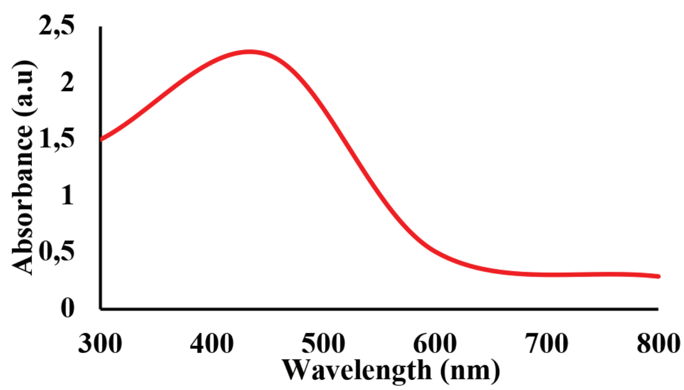


Figure 1- UV-VIS measurement of silver nanoparticle

AgNPs peaked between 400 nm and 800 nm. Both color change and UV-VIS results show that AgNPs were synthesized (Pugazhendhi et al. 2018).

3.2. SEM and SEM EDX analysis of AgNPs synthesized from Nasturtium officinale

The SEM images of AgNPs synthesized from *Nasturtium officinale* are given in Figure 2. The SEM images of AgNPs showed small shapes between 1 µm and 10 µm.

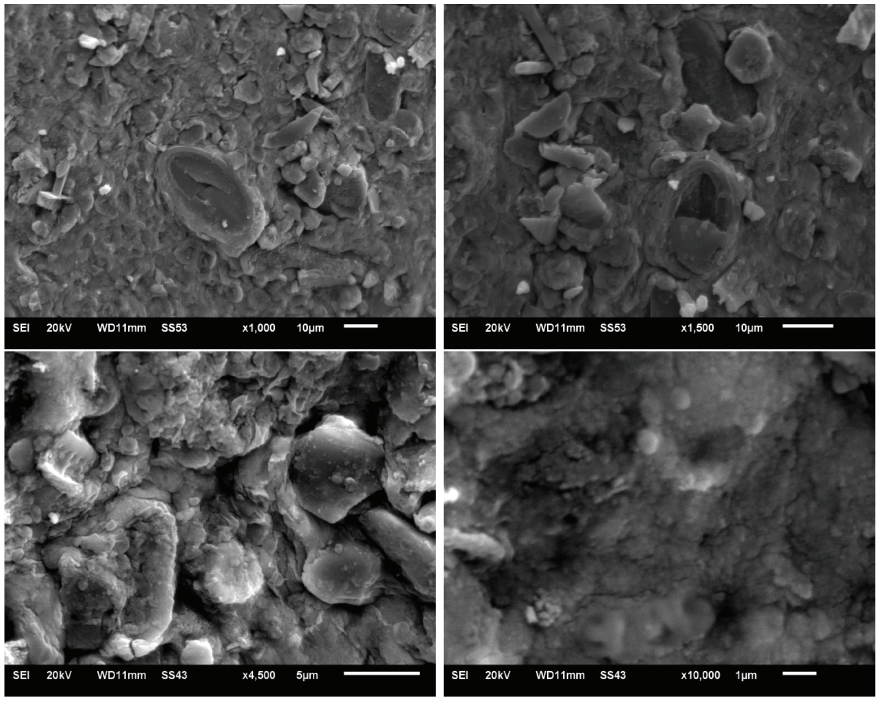
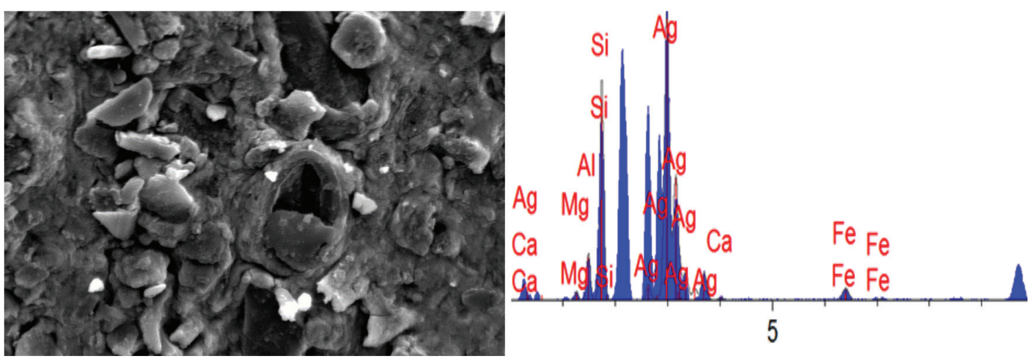


Figure 2- SEM images of AgNPs

The SEM EDX images and a graph of AgNPs are given in Figure 3. The SEM EDX images of AgNPs gave an image similar to small pebbles of different sizes.



Elt.	Line	Intensity (c/s)	Error 2-sig	Conc	Units
Mg	Ka	21.51	2.693	0.767	wt.%
Al	Ka	114.94	3.877	3.388	wt.%
Si	Ka	563.51	6.857	14.667	wt.%
Ca	Ka	84.54	3.866	2.791	wt.%
Fe	Ka	50.13	3.337	2.327	wt.%
Ag	La	901.11	8.940	76.061	wt.%
				100.000	wt.%

Figure 3- SEM EDX image and graph of AgNPs

3.3. XRD analysis of the AgNPs synthesized from Nasturtium officinale

Scanning angle 3-90, scanning speed 10°/min, 2θ (2 theta) versus intensity spectrum graph is given in Figure 4. The 2θ angle was found to be 32.312, 46.30, 67.34, and 76.80. The crystal structure of silver nanoparticles was approved through an XRD analysis as shown in Figure 4. Also, in addition to the Bragg peaks, additional peaks were monitored at 27.83, 54.86, and 57.55. These peaks are the result of organic compounds in charge of the reduction of silver ions (Roopan et al. 2013). The resulting XRD pattern is consistent with previous studies (Kumar et al. 2014; Ibrahim 2015).

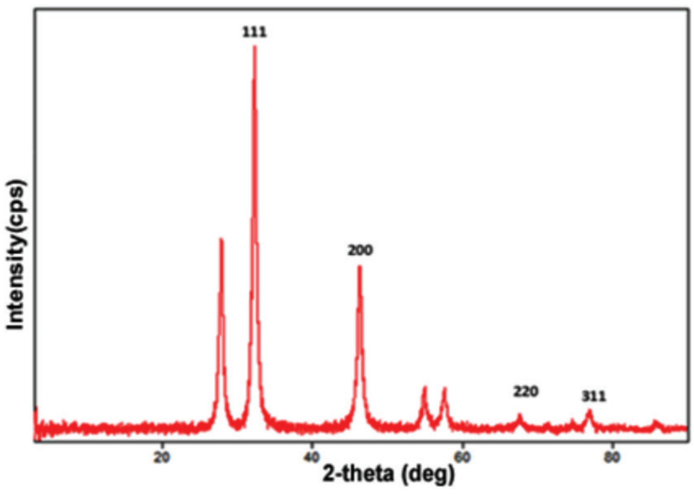


Figure 4- XRD analysis result of AgNPs synthesized from *Nasturtium officinale*
cps: Counts per second, 2-theta: Diffraction angle

3.4. FTIR analysis of the AgNPs synthesized from Nasturtium officinale

The FTIR results of AgNPs nanoparticle gave peaks between 3672-668 (Figure 5). The bands 3672.41, 3187.13, 2926.72, 2323.27, 1995.70, 1761.37, 1633.62, 1473.30, 1398.82, 1130.05, 1020.40, 782.08, 668.78 cm^{-1} vibrated. The vibration of the nanoparticle at 3672 is of the O-H bond, at 3317 of the C-H bond, at 2323.27 the vibration of the C≡C- bond or C≡N bond, the 1995-1633 bands of the C=O bond, at 1130 band of the C-O bond and 782 band of C-H bond (Ciursă et al. 2021; Wang et al. 2022). The FTIR spectra of silver nanoparticles synthesized using *Nasturtium officinale* showed distinct peaks representing functional groups responsible for Ag^+ reduction. These functional groups can bind with reduced Ag metal to stabilize nanoparticles (Adebayo-Tayo et al. 2022).

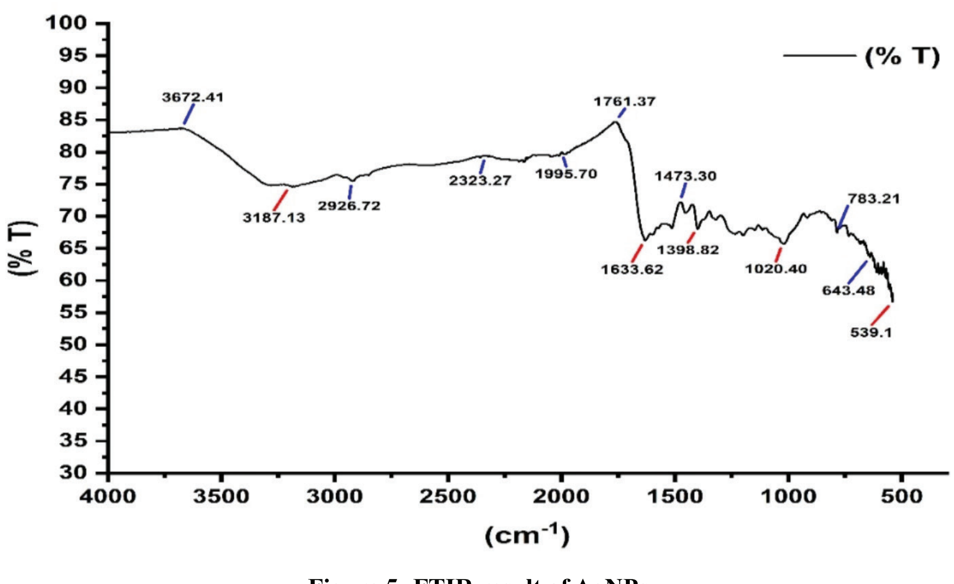


Figure 5- FTIR result of AgNPs
% T: % Transmittance, cm^{-1}: Wavelength

3.5. UV-VIS analysis of ZnNPs synthesized from Nasturtium officinale

The UV-VIS spectroscopy plot of ZnNPs is given in Figure 6.

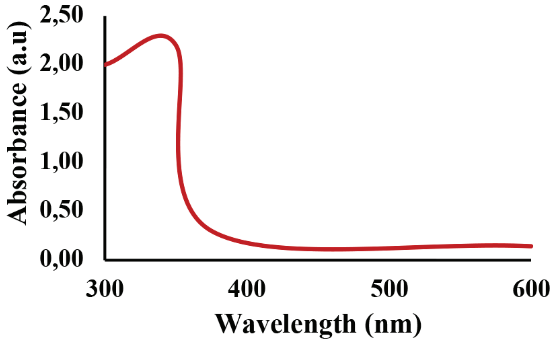


Figure 6- UV-VIS measurement results of zinc nanoparticles

3.6. SEM and SEM EDX analysis of ZnNPs synthesized from Nasturtium officinale

The SEM images of ZnNPs synthesized from *Nasturtium officinale* are given in Figure 7.

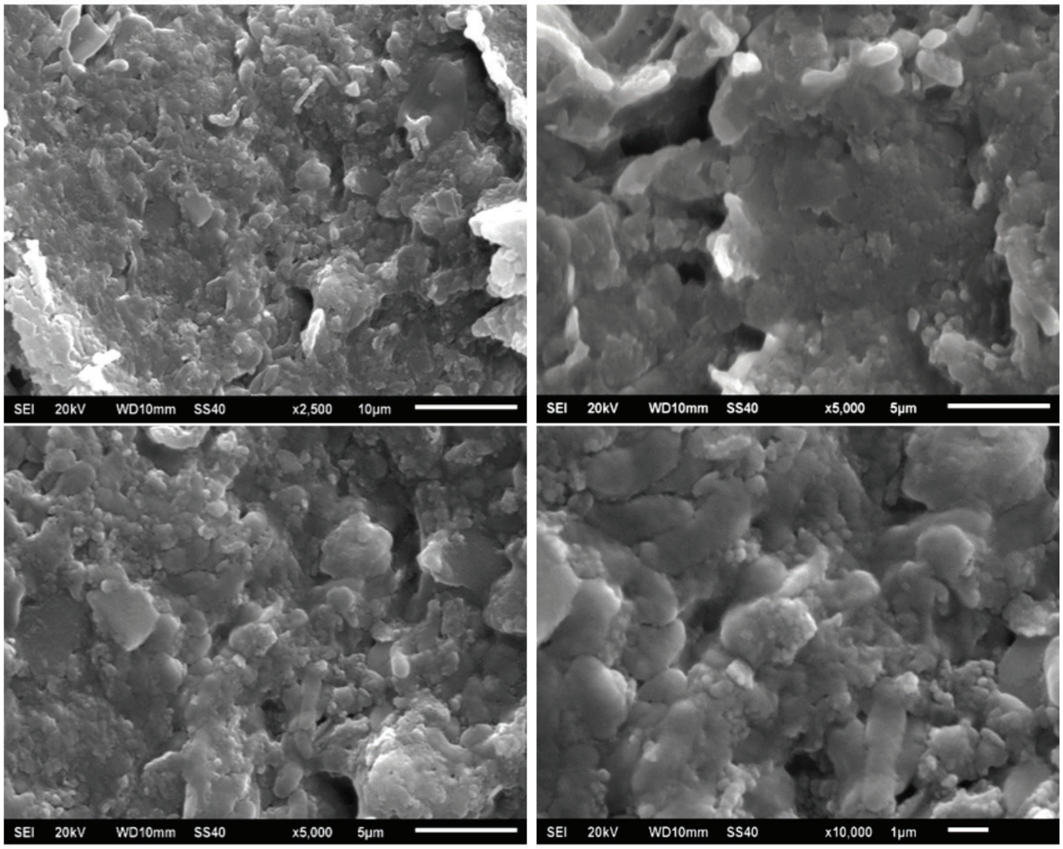
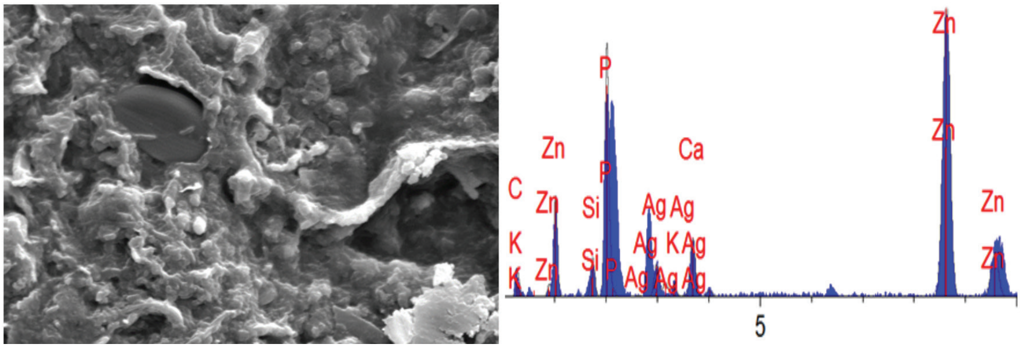


Figure 7- SEM images of ZnNPs

The SEM EDX images and a graph of ZnNPs synthesized from *Nasturtium officinale* are given in Figure 8.



Elt.	Line	Intensity (c/s)	Error 2-sig	Conc	Units
C	Ka	32.87	3.460	10.039	wt.%
Si	Ka	60.38	5.445	1.941	wt.%
P	Ka	408.63	10.059	11.767	wt.%
K	Ka	15.22	4.925	0.352	wt.%
Ca	Ka	114.98	6.361	2.689	wt.%
Zn	Ka	847.70	13.667	68.344	wt.%
Ag	La	61.32	5.770	4.868	wt.%
				100.000	wt.%

Figure 8- SEM EDX image and graph of ZnNPs

When the SEM and SEM EDX images of the synthesized ZnNPs are examined, it is seen that the nanoparticles form bubble-like shapes.

3.7. XRD analysis of the ZnNPs synthesized from Nasturtium officinale

The XRD result of ZnNPs synthesized from *Nasturtium officinale* is given in Figure 9.

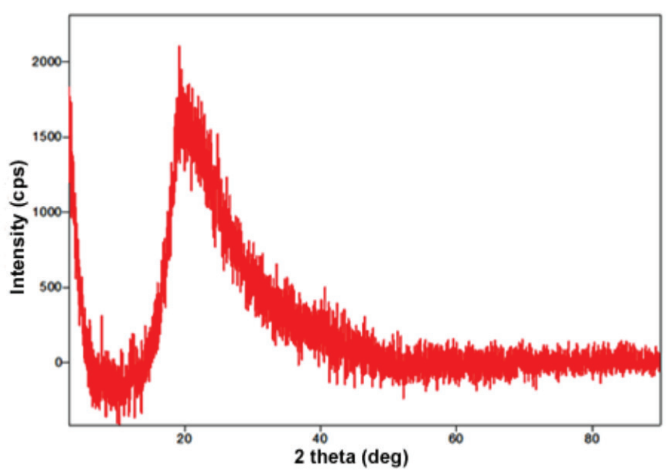


Figure 9- XRD analysis result of synthesized ZnNPs
cps: counts per second, 2-theta: diffraction angle

When the XRD analysis result of synthesized ZnNPS was examined, it was seen that composite material was synthesized in nanostructure (Rangelova et al. 2022).

3.8. FTIR analysis of the ZnNPs synthesized from Nasturtium officinale

When the FTIR result of ZnNPs was examined, it was observed that the nanoparticle gave a peak between 3275-628 (Figure 10). It gave peaks for many identifiable organic compounds. The bands 3275-2924 indicate the presence of a C-H bond, the band 2146 the presence of C≡C bond or C≡N bond, the band 1830 the presence of a C=O bond, the 1622-1579-1480 bands the presence of a C=C bond (double bonds), the 1225 band the presence of C-O bond. In addition, the 1013 band indicates the presence of the C-O bond, and the presence of the 838 band indicates the presence of the C-H bond (Hosseini et al. 2020; Ciursă et al. 2021; Wang et al. 2022).

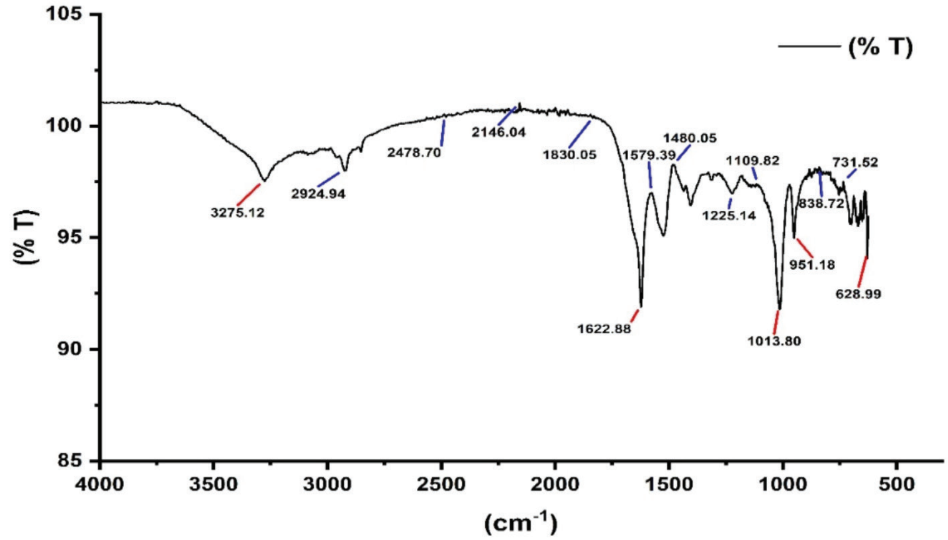


Figure 10- FTIR result of ZnNPs
% T: % Transmittance, cm^{-1}: Wavelength

3.9. Antimicrobial effects of AgNPs and ZnNPs nanoparticles

The antimicrobial analysis results of the prepared nanoparticles and the erythromycin antibiotic are given in Table 1 and Table 2.

Table 1- Antimicrobial activity of AgNPs and ZnNPs nanoparticles

Microorganisms	*Erythromycin (15 µg)*	*AgNPs (20 mg/mL)*	*ZnNPs (20 mg/mL)*
K. pneumoniae	16.43±0.02	11.9±1.3	8.14±1.07
E. coli	14.34±1.4	11.62±0.9	-
S. aureus	18.98±0.5	16.52±2.4	9.82±1.2
P. aeruginosa	12.63±0.02	11.18±0.7	-
S. pyogenes	20.7±0.1	11.64±1.5	-
C. albicans	15.20±1.1	12.18±0.3	9.55±0.42

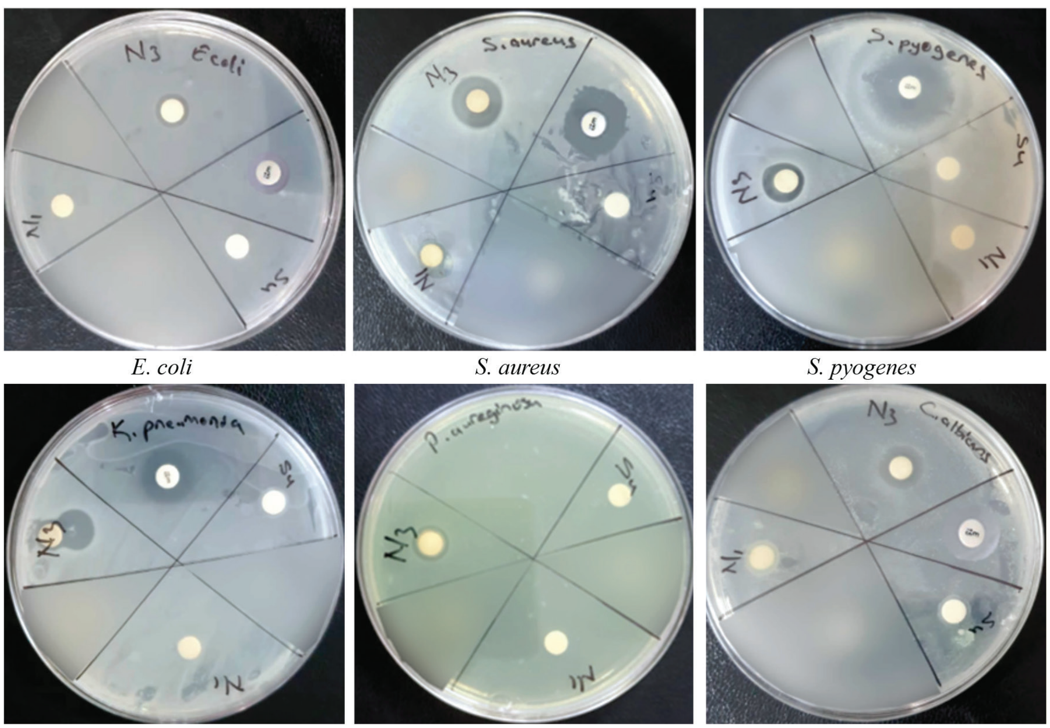
Inhibition zone diameter ± standard deviation (mm)

Table 2- Bacteria and fungus % inhibition values of AgNPs and ZnNPs according to erythromycin antibiotic

Microorganisms	*% inhibition for AgNPs*	*% inhibition for ZnNPs*
K. pneumoniae	72.43	49.54
E. coli	81.03	
S. aureus	87.04	51.74
P. aeruginosa	93.98	
S. pyogenes	56.23	
C. albicans	80.13	62.83

According to the results, watercress nanoparticles synthesized with Zn and Ag have antimicrobial properties.

In experiments investigating the antimicrobial activities of different nanoparticles prepared with watercress, a marsh plant, AgNPs showed the most effective antimicrobial activity (Figure 11). Silver is a non-toxic substance capable of killing many disease-causing microorganisms (Jeong et al. 2005; Ibrahim 2015). Due to the antimicrobial properties they exhibit, it is predicted that silver nanoparticles can be widely used as antimicrobial agents in the future (Rai et al. 2009; Ibrahim 2015). AgNPs synthesized from watercress was highly effective on both gram-negative bacteria (*E. coli, K. pneumoniae, P. aeruginosa*) and Gram-positive bacteria (*S. aureus, S. pyogenes*) as well as fungi (*C. albicans*). The strong antimicrobial effect of synthesized AgNPs showed that *Nasturtium officinale* could be a desirable choice for the synthesis of antimicrobial nanoparticles and biotechnological products. Zn, which is an important mineral in terms of health that participates in the structure of many important enzymes, is one of the prominent minerals in nanoparticle synthesis. The ZnNPs nanoparticle showed an inhibition effect on *K. pneumoniae* and *S. aureus* bacteria and fungi (*C. albicans*). However, it was observed that the antimicrobial effect of ZnNPs synthesized from *Nasturtium officinale* was less than that of AgNPs (Figures 11, 12). The effect of silver and zinc nanoparticles, synthesized with *Nasturtium officinale* on *S. aureus* or *S. aureus* and *E. coli,* respectively, have previously been studied (Sadeghi 2014; Bayrami et al. 2019). However, in this study, the effects on 6 microorganisms were examined and gave very effective results which show that *Nasturtium officinale* is highly effective in the synthesis of antimicrobial products.



E. coli *S. aureus* *S. pyogenes*

K. pneumoniae *P. aeruginosa* *C. albicans*

Figure 11- Antimicrobial effects of AgNPs and ZnNPs nanoparticles
(N_1= ZnNPs, N_3= AgNPs, E15: Erythromycin)

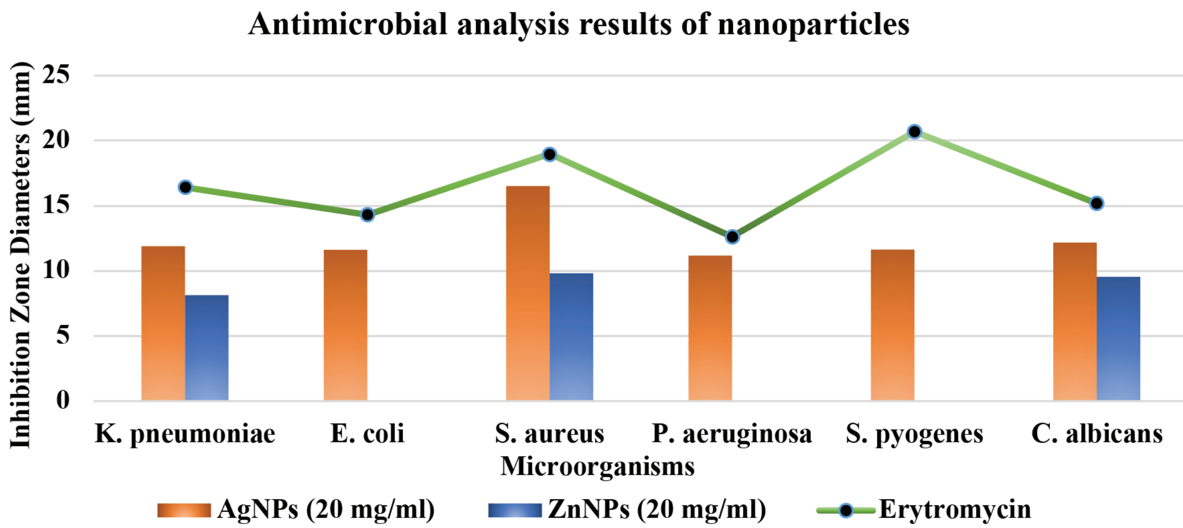


Figure 12- Antimicrobial activity graph of AgNPs, ZnNPs, and erythromycin (mm)
X: Inhibition zone diameters (mm), Y: Bacteria, fungi, and nanoparticles

4. Conclusions

It has been shown that plants that grow spontaneously in nature can be used efficiently in the synthesis of antimicrobial agents. Silver nanoparticles showed a high antimicrobial effect. *Nasturtium officinale* is an extremely suitable plant for the synthesis of nanoparticles with antimicrobial properties. In addition to being natural and inexpensive, antimicrobial agents that are easy to synthesize can have broad-spectrum antimicrobial effects.

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