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Green synthesis, characterization, antimicrobial and antioxidant activities of zinc oxide nanoparticles using *Helichrysum arenarium* extract

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Abstract

Active substance production at the nano-level attracts attention in the scientific world due to its wide application area. Different methods are used for the biosynthesis of nanoparticles. Recent studies have focused on non-toxic, environmentally friendly synthesis methods. Nanoparticles obtained by green synthesis using various biological elements such as plants, microorganisms and proteins have taken part in many scientific studies. Plants, which have an important potential in active ingredient production, are highly preferred in nanoparticle production. Scanning Electron Microscope and Energy Dispersive X-Ray Analysis (SEM / SEM-EDX), Fourier Transform Infrared Spectroscopy (FT-IR), X-Ray Diffraction (XRD) and Ultraviolet visible light absorption Spectroscopy (UV-vis) techniques were used for the structural and morphological characterization of Zn nanoparticles obtained by green synthesis using *Helichrysum arenarium* plant extract and ZnO.The antioxidant capacity of Zn NPs/Ha structures was determined by performing the DPPH test. Antimicrobial effects of zinc nanoparticles on six different pathogens (*Bacillus cereus* ATCC 10876, *Escherichia coli* ATCC 25952, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATTC 29213, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATTC 90028) were investigated. As a result of this studies, it has been observed that it has an inhibitory effect against some pathogen microorganisms. It has also been found that its antioxidant content is at a significant level.

Keywords: Zinc Oxide, Nanoparticle, Antimicrobial, *Helichrysum arenarium*

Introduction

In recent years, the using of nano and microstructures in science and technology has been increasing (Herlekar et al., 2014). It is thought that the nanomaterials produced by Green synthesis can be used as anti-cancer agents and pharmaceutical agents (Hanan et al., 2018; Hay et al., 2017; Dipankar & Murugan, 2012; Bupesh et al., 2016). Understanding the biocompatibility of nanoparticles is a necessary step for biomedical applications (Das et al., 2016). It has quickly gained popularity in different industries such as nanotechnology, bioenergy, and agricultural systems (Rai et al., 2018). Synthesis of metal nanoparticles using plants has been an important way to overcome the limitations of traditional synthesis approaches such as physical and chemical methods (Rajeshkumar & Bharath, 2017; Wu et al., 2017). Research in nanotechnology has yielded environmentally and biologically safe results. Plant-based synthesis of metal nanoparticles was chosen as the best strategy because of its environmental friendliness and ease of synthesis (Khan et al., 2019). *Helichrysum arenarium* (L.) plant is widely available in Asia and Europe. This plant that has been used in traditional therapy, it has a good antioxidant content used in cholecystitis and bile treatments (Tepe et al.,

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2005). It has been determined that the essential oils obtained from the flowers of the *Helichrysum arenarium* plant have an important antimicrobial potential (Rančić et al., 2005). Zinc is an important micro element for humans found in protein and enzymes (Prasad, 2014). Zinc oxide (ZnO) nanoparticles have properties such as antimicrobial and photocatalytic activity (Miri et al., 2019). Zinc Oxide-derived nanoparticles have become a new treatment thanks to their chemical and thermal resistance (Wu, 2019). ZnO nanoparticles obtained by Green synthesis have been shown to inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* pathogens (Ansari et al., 2020).

In our study, nanoparticles were obtained by green synthesis using *Helichrysum arenarium* plant and ZnO. Scanning electron microscopy (SEM/SEM-EDX) image was obtained for the structural and morphological characterization of ZnO nanoparticles. At the same time, FT-IR analysis, X-Ray Diffraction method (XRD) and UV-vis spectrophotometer analyzes were performed to demonstrate the reliability of the obtained nanoparticles. Antimicrobial effects of ZnNPs/Ha structures on six different pathogens were investigated by performing antioxidant content testing.

Materials and Methods

Preparation of plant extract

Helichrysum arenarium plant collected from the mountainous area of Van in Turkey was washed in the laboratory. After drying for 7 days at room temperature, it was pulverized with a grinder. 100 g of the ground sample was taken and left to boil at 80 ° C for 10-12 minutes with 500 mL of distilled water. It was cooled after the color change occurred. Filtration was performed by using Whatmann No: 1 filter paper and the obtained extract was stored at 4 °C (Gün et al., 2011).

Synthesis of ZnO nanoparticles

For the synthesis of ZnO nanoparticles, 1 mM 500 ml of ZnO solution and 100 ml of *Helichrysum arenarium* plant leaf extract were reacted in a 1000 ml flask at room temperature. Color changing in the solution was observed with in 25-40 minutes with the reduction of zinc ions. The resulting solution was centrifuged at 10.000 rpm for 5 minutes and the liquid phase remaining in the upper phase was discarded. The solid phase was washed several times. The obtained particles (Zn NPs/Ha) were left to dry for 72 hours at 45-50 °C in the oven. It was stored at 4 °C for characterization and analysis (Joseph & Mathew, 2015).

Characterization of Zn NPs/Ha

Topography of the samples was taken for scanning electron microscopy (SEM) imaging. In addition, the density of the metal was measured by SEM-EDX (SEM, Zeiss Smart EDX). The crystal structure of Zn NPs / Ha was obtained using XRD technique (Panalytical Emperian diffractometer, 40 mA, 40 kV, k 1.54056). Characteristic absorption values for zinc nanoparticles were investigated using UV-Vis (PEL 750 instrument (measurement range 250-800 nm wavelength)). FT-IR analysis was measured in the wavelength range of 500-4000 cm-1 using the Perkin Elmer Spectrum Two Spectrometer (Aiken & Finke, 1999).

Antioxidant activity (DPPH)

The DPPH quenching activity of Zn NPs/Ha was calculated using the previously method (Blois, 1958; Koçak et al., 2020). BHA and BHT were used as positive controls in this method. The experiment was performed using 0.1 mg/ml DPPH methanol solutions. DPPH and extracts with the same ratio were prepared in 5 different concentrations of 25, 50, 100, 200 and 250 µg/ml. 3 ml of Zn NPs/Ha extract and positive control were taken and DPPH solution was added on them. The mixtures formed in the tubes were left to incubate for 30 minutes in the dark and at room temperature. At the end of this period, absorbance values were measured at 517 nm.

% I = [(Akontrol-Aample) / Akontrol] × 100

According to the result of this process, a graph of Zn NPs/Ha concentration versus increasing DPPH ethanol concentration was obtained.

Antimicrobial Activity

The antimicrobial activity of *Helichrysum arenarium* plant and zinc nanoparticles was investigated by disk diffusion method (Prabhu et al., 2010). *Bacillus cereus* ATCC 10876, *Escherichia coli* ATCC 25952, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATTC 29213, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATTC 90028 were used as pathogens. Test microorganisms were obtained from Van Yüzüncü Yıl University, Faculty of Science, Department of Biology. Müller Hinton medium was used in this study. Plant extract and Zn NPs/Ha 25 µL were absorbed on blank discs and dried at room temperature. Then the discs and Neomycin (10 µg) were placed in the medium with the positive control group. The prepared petri dishes were kept in the oven for 24 hours at 36.5 °C for incubation. Then the zone measurements were made and the images were recorded.

Results and Discussion

Characterization of zinc nanoparticles

It was determined that ZnNPs subjected to electron by scanning electron microscope (SEM) have a shallow and spherical structure (Figure 1.). The SEM/EDX spectrum of ZnNPs shows the peaks of zinc, carbon, calcium, chlorine and oxygen elements (Figure 1.) (Elumalai & Velmurugan, 2015). The UV-Visible absorbance spectrum used in the studies should show the nanoparticle formation In this context, it was observed that the ZnO nanoparticle peaked at 370 nm (Figure 2). It was observed that ZnO nanoparticles were formed by using *Helichrysum arenarium* extract. It was observed that Zinc oxide analyzed using UV-Vis spectroscopy formed a strong surface plasma peak at 370 nm wavelength (Büyük and Ilıcan, 2018). However, it was seen that the peak formed disappeared because Zn NPs/Ha was reduced to Zn^{+2}- Zn^{0} due to the transition between the electrons (Figure 2). XRD technique was used to determine the crystal structure of zinc nanoparticles. X-ray diffraction (XRD) of synthesized zinc nanoparticles was used to verify the crystal structure of the particles. Figure-3 shows a representative XRD model of zinc nanoparticles synthesized with *Helichrysum arenarium* plant extract after complete reduction of zinc. In the present study, it is seen in Figure 3 that ZnONPs is crystallized as hexagonal wurtzite form. XRD results appear to be in line with studies in the literature. The XRD results obtained are in parallel with similar studies in the literature. Considering the spectrum, different peaks are seen (Figure 3). It has been determined that Zn NPs have different refractive peaks as 32.05 ° 37.45 ° 47.23 ° and 66.22 ° versus Zn (111), Zn (200), Zn (220), and Zn (311) planes (Anvekar et al., 2017). Figure 4 shows peaks formed by functional groups (glycine, octasiloxane, glycolic acid, flavones, coumarin, glycoside) in the structure of *Helichrysum arenarium*, which is the current plant in the study, in the range of 800-1500 cm^{-1} (Figure 4). It is thought that the peaks in between 3500-2700 cm-1belong to many organic components and the sharp peak at 2900-3000 cm-1 belongs to the C-H organic component (Xiong et al., 2009).

Antioxidant activity (DPPH)

Antioxidants can prevent cell damage by clearing the damaging molecules called free radicals in our cells. In our current study, by determining the antioxidant properties of ZnNPs created using ZnO, the quenching activity of DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radical was determined. DPPH radical is a commercially available free radical and a safe molecule in total antioxidant calculations. This radical is a purple colored solution and provides maximum absorbance at 517 nm (Yu et al., 2002). It is observed that the purple color of DPPH radical, which reacts with antioxidant components, decreases over time and its absorbance value decreases. In our current study, Zn NPs/Ha clusters were compared with the positive control BHA and BHT (Figure 5.). DPPH radical quenching activities for positive control BHA and BHT at 100 µg/ml concentration, respectively; While it is 94.155-89.480%, this value for Zn NPs/Ha is 79.871%. Compared to many studies, Zn NPs/Ha appears to be a good antioxidant (Nunes et al., 2018; Suresh et al., 2015).

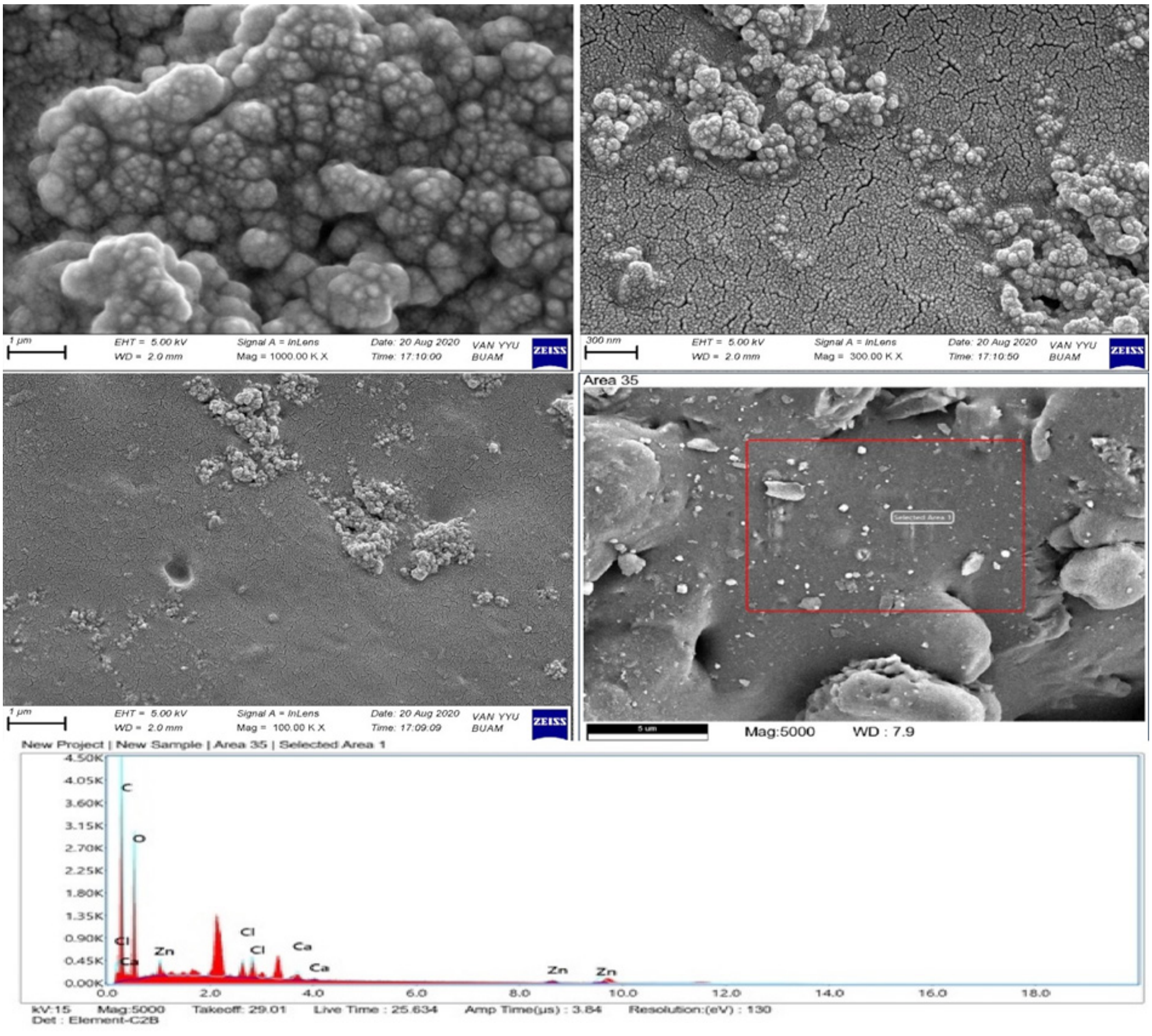

Figure 1. Scanning electron microscope (SEM) and SEM / EDX spectrum image of Zn NPs/Ha.

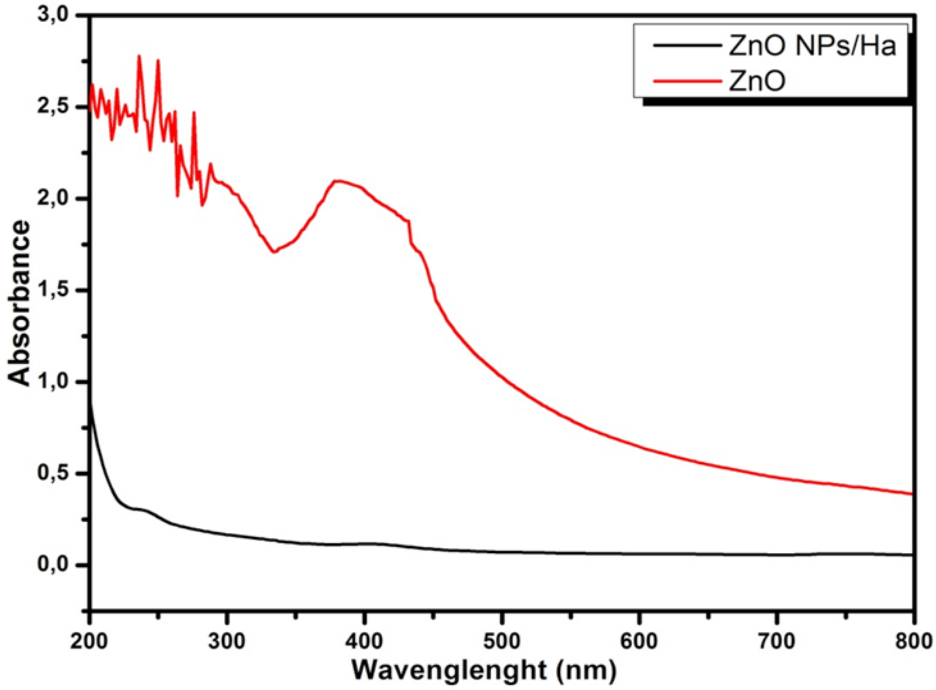

Figure 2.UV-vis spectra of ZnO and Zn nanoparticles

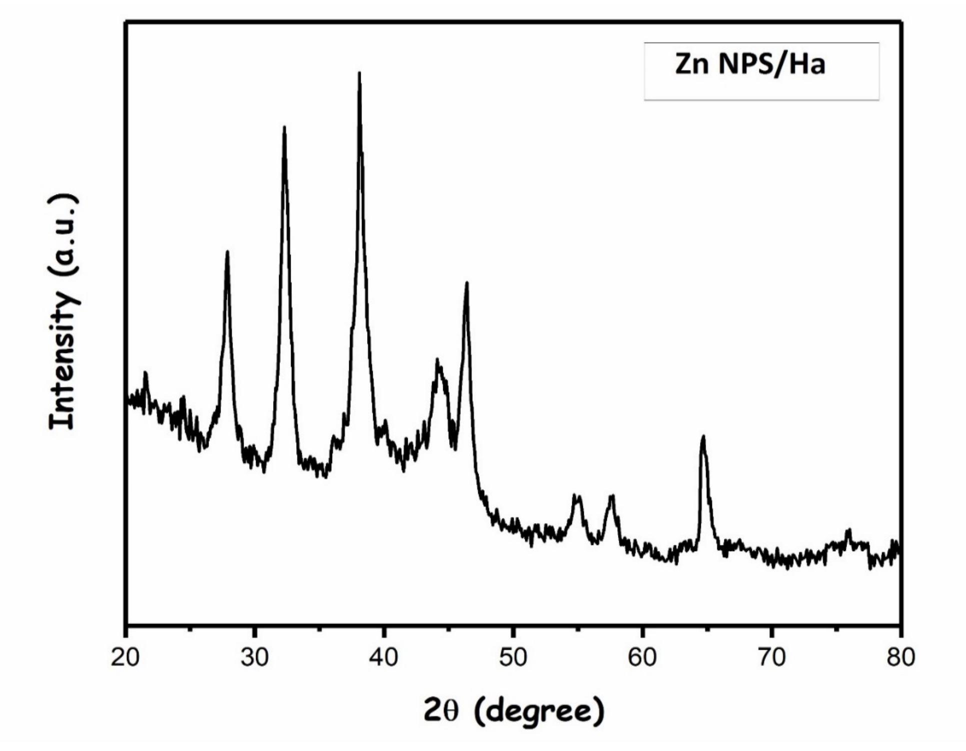

Figure 3. XRD graph of Zinc Oxide nanoparticles

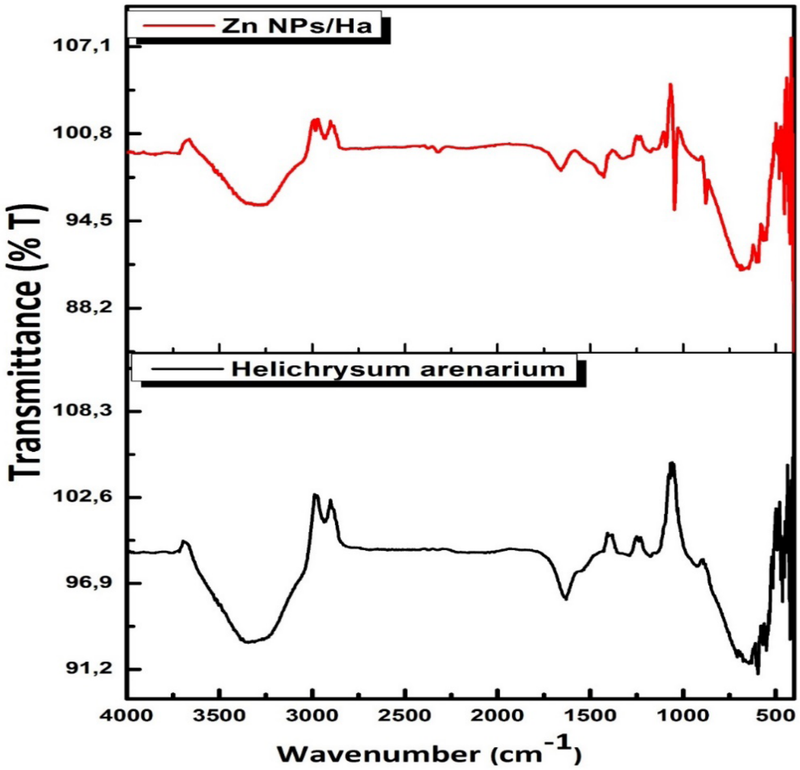

Figure 4. FT-IR spectra of *Helichrysum arenarium* and Zn NPs/Ha samples

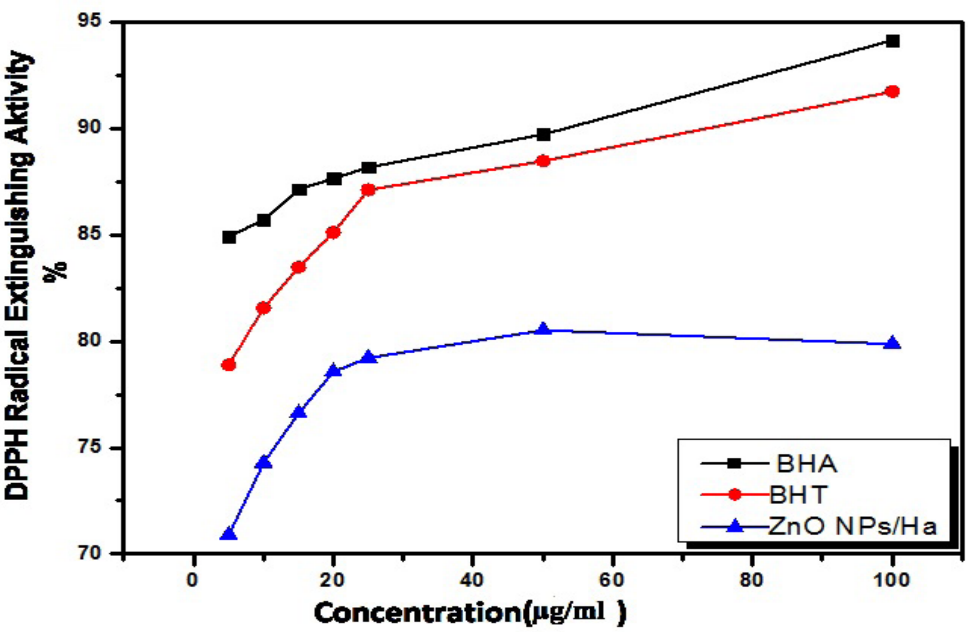

Figure 5. DPPH Radical activity of ZnO nanoparticles.

Antimicrobial Activity

According to the obtained data, it was determined that *Helichrysum arenarium* plant extract and Zn-NPs/Ha clusters were effective against pathogens. In the antibacterial activity measurements, it was determined that synthesized ZnO nanoparticles provided bacterial activity inhibition in *E. coli* and *S. aureus* (Erdoğan et al., 2019). ZnONPs are known to inhibit the growth of *Campylobacter jejuni, Salmonella enterica, Escherichia coli, Vibrio cholerae* and *Staphylococcus aureus* bacteria (Sirelkhatim et al., 2015). It was observed that extract and ZnO nanoparticles formed an inhibition zone varying between 8.15-11.15 mm against pathogenic microorganisms. Antimicrobial activity results are given in Table 1, the zone rates formed are in Figure 6 and some images obtained as a result of disk diffusion are given in Figure 7.

Table 1. Antimicrobial activity results.

Test Microorganisms	Inhibition zones (mm)		
Bakteria	Extract	Zn NPs/Ha	Neomycin
Bacillus cereus ATCC 10876	9±1.5	9±3.0	20±1.0
Bacillus subtilis ATCC 6633	9±1.0	8±1.5	22±1.5
Escherichia coli ATCC 25952	10±1.0	9±2.5	14±2.5
Pseudomonas aeruginosa ATCC 27853			
Staphylococcus aureus ATTC 29213	10±2.0	9±2.0	17±2.5
Fungus			
Candida albicans ATTC 90028	11±1.5	9±1.0	21±3.0

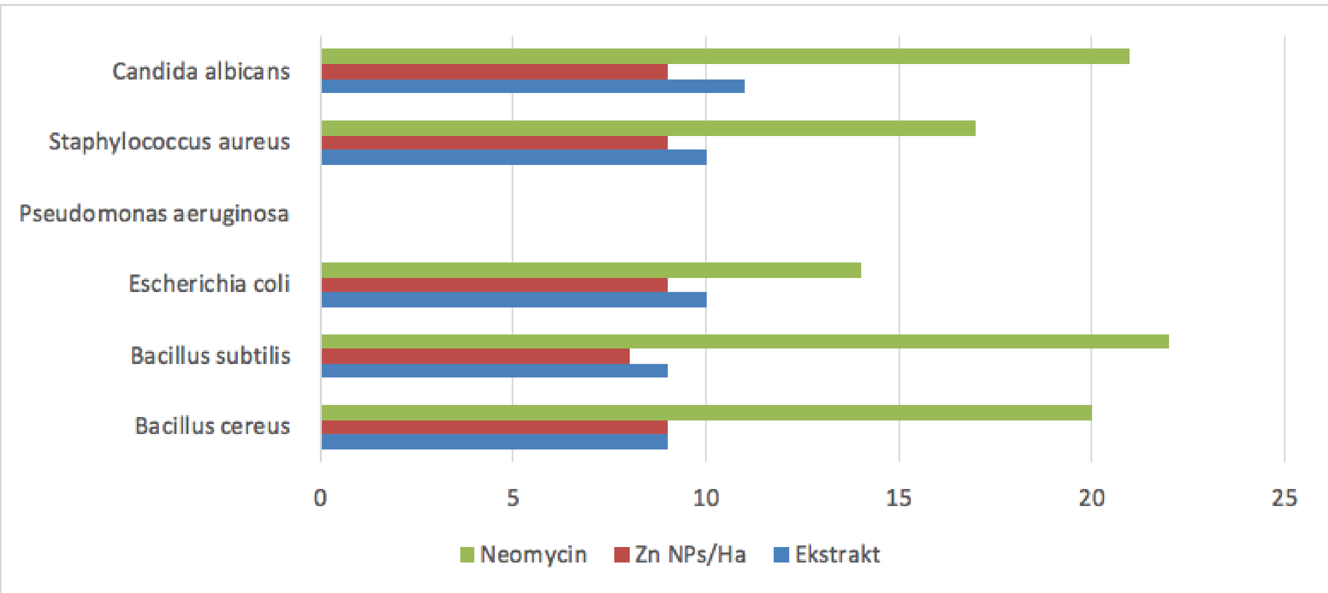

Figure 6. Zone rates resulting from antimicrobial activity

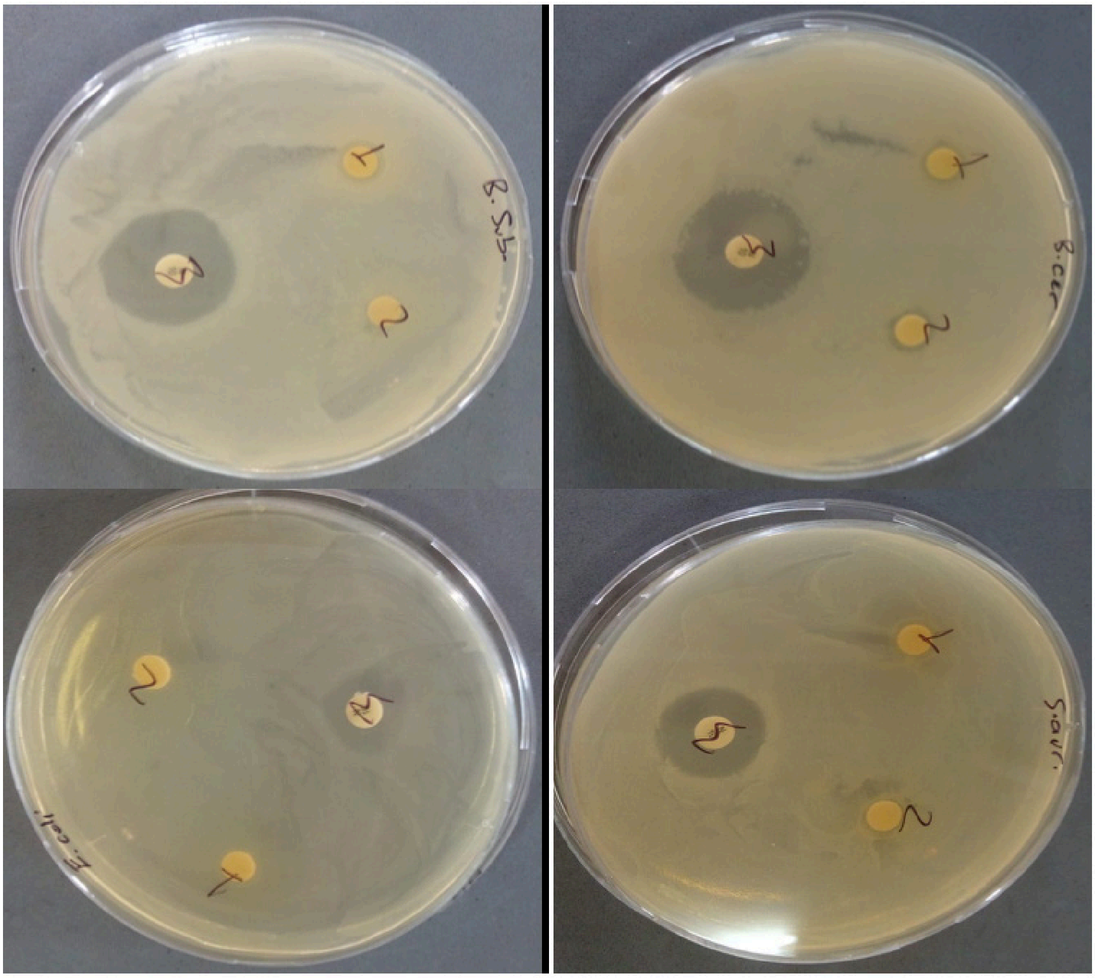

Figure 7. Some images obtained as a result of disk diffusion

Conclusion

Considering the radical quenching activity of ZnO-NPs obtained by using *Helichrysum arenarium* plant, it is understood that it is a powerful antioxidant. In addition, considering the zones formed against harmful microorganisms, it has been determined that it has an antimicrobial effect. It is thought that it can be used as an antimicrobial agent in the fields of medicine and pharmacology by conducting more comprehensive content analysis.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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