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Antimicrobial activities of silver nanoparticles synthesized from *Helichrysum italicum* (Roth) G. Don

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Abstract

In our study, the ability to produce silver nanoparticles (AgNP) by green synthesis method using *Helichrysum italicum* (Roth) G. Don. plant was investigated. The color of the extract changed from yellow to brown, proving the presence of AgNPs. Transmission electron microscope (TEM), scanning electron microscopy (SEM), and Energy-dispersive spectra (EDS), FT-IR and Uv-Vis analysis were used to characterized AgNPs. AgNPs ranged from 20 nm to 40 nm in size and were mostly spherical and cubic structures. The antimicrobial potential of AgNPs was tested against seven bacterial and one yeast cultures by disc diffusion and minimum inhibitory concentration (MIC) methods. AgNPs significantly inhibited all test cultures. The highest antimicrobial activities of plant extract and AgNP were found against *Proteus vulgaris* ATCC 13315.

Keywords: *Helichrysum italicum* (Roth) G. Don.; Silver nanoparticle; Antimicrobial.

1. Introduction

Nanoparticles refer to particles that are 100 nm in size or smaller. Unlike bulk materials, nanoparticles exhibit unique physical, chemical, electronic, magnetic, optical, and biological properties owing to their nano-scale structures [1]. They also facilitate the advancement of novel approaches in medical applications such as the creation of antimicrobial agents, drug delivery systems, disease diagnosis, and treatment [2]. Nanoparticles can be synthesized from various metals such as silver, gold, palladium, iron, copper, lead, titanium, lithium, zinc, cerium, and metal oxides using the green synthesis method. In recent years, significant research has focused on synthesizing silver nanoparticles (AgNPs) using different plant and fruit extracts [3-4]. One advantage of utilizing plant and fruit extracts is the formation of stable nanoparticles that resist molecular aggregation and can be stored for extended periods. Studies have shown that AgNPs prepared via synthetic methods demonstrate effective antimicrobial properties [5]. AgNPs with smaller particle sizes are particularly promising for developing new pharmaceuticals, antimicrobial agents, anticancer drugs, and antibiofilm agents [6].

We aimed to synthesize AgNPs using the flowers of *Helichrysum italicum* (Roth) G. Don., which belongs to the Asteraceae family. *H. italicum*, also known as "*ölmez otu*", is an aromatic shrub that emits a strong and long-lasting fragrance, similar to the scent of curry, with small yellow flowers [7]. In the Mediterranean region, *H. italicum* has been used in folk medicine for centuries for the treatment of various ailments including toothache, digestive disorders, cough, colds, laryngitis, skin diseases, alopecia, dermatomycosis, liver and gall disorders, bronchitis, insomnia, headache, and herpes [8]. Scientists have demonstrated its potent anti-inflammatory [9], antioxidant [10], antimicrobial [11-12-13], antiviral

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[14], and antiproliferative [15] activities. Therefore, this plant's numerous beneficial effects on human health have led to its wide application in various industries such as pharmaceuticals, food, perfumery, and cosmetics.

The main aim of this study is to biologically synthesize AgNPs using *H. italicum*, characterize the synthesized product, and investigate the antimicrobial activities of the AgNPs.

2. Materials and methods

2.1. Experiment Materials

The dried *H. italicum* plant, acquired from an herbalist, was mechanically ground. The plant material was then extracted for 60 minutes with 100 mL of pure water at 65-70°C using a heated magnetic stirrer, and subsequently filtered through filter paper to eliminate impurities.

2.2. Green Synthesis of AgNP

For AgNP synthesis, the previously prepared extract of *H. italicum* flowers was mixed with a 1 mM $AgNO_3$ aqueous solution in a 1:9 ratio in a 1000 mL flask under ambient conditions for 30 minutes. The resulting dark-colored solution, formed through the reduction of silver ions, was centrifuged at 10.000 rpm for 60 minutes to remove the supernatant. The remaining pellet was washed three times with distilled water. The pellets were then transferred to sterile petri dishes and dried in an oven at 65°C for 48 hours [16].

2.3. Characterization of AgNPS

The size, shape, and surface morphology of AgNPs, as well as their chemical analysis, were performed using Transmission electron microscope (TEM), scanning electron microscopy (SEM), and Energy-dispersive spectra (EDS) techniques at Atatürk University Eastern Anatolia High Technology Application and Research Center (DAYTAM) through service procurement.

UV-Vis spectroscopy (UV—Vis) provides information about the structure, size, stability, concentration, and aggregation of nanoparticles [17]. The biosynthesis of AgNPs in distilled water solution (1 mg/mL) was monitored by measuring UV-Vis spectra. The UV-Vis spectrum was recorded by periodic scans of optical absorbance between 200 nm and 800 nm using a UV-Visible double-beam spectrophotometer.

FT-IR spectroscopy is utilized to determine the structure and functional groups associated with nanoparticles and biological extracts based on the wavelength of light [18]. FT-IR analysis was conducted to identify the reducing agent involved in the synthesis of AgNPs. FT-IR spectra of extracts and AgNPs (KBr disk) were recorded and analyzed in the range of 4000-400 cm^{-1} with a resolution of 1 cm^{-1}.

2.4. Antimicrobial Activity Assays

Disk diffusion and microdilution (MIC) methods were used to determine the antimicrobial effect of AgNPs on bacteria and yeast cultures [19]. In antimicrobial studies, a total of 8 microorganisms, *Escherichia coli* NRRL B-3704, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Staphylococcus haemolyticus* ATCC 43252, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 13315, *Acinetobacter baumanii* ATCC19066, and *Candida albicans* ATCC 10231 were used. Test cultures were suspended on Mueller Hinton Agar (MHA), sterile discs were placed on the medium, and 20 µL of AgNP was added to each disc. After 24 hours of incubation, zone formation around the discs was examined. The MIC testing was performed in a 96-well round-bottom microtiter plate using the standard broth microdilution method. Test cultures and AgNPs prepared with McFarland 0.5 turbidity standard were then added for each culture and incubated overnight at 37°C. The lowest concentration value without microbial growth was accepted as the MIC value [20]. Penicillin, streptomycin, and nystatin were used as positive controls.

3. Result and discussion

The initial prominent observation of nanoparticles in our study is the color change that occurred. In our study, Ag ions reduced by *H. italicum* were observed to change the extract's original yellow color to a brown hue (Figure 1).

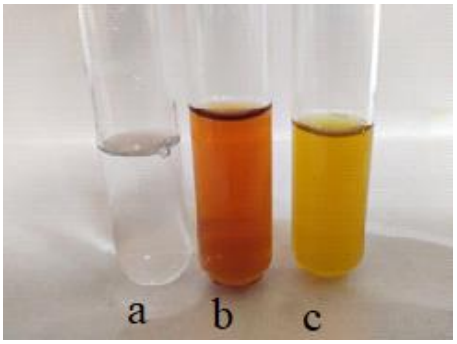

Figure 1 Color changes of synthesized AgNP. (a) Extract of $AgNO_3$ (b) Synthesized AgNP (c) Aqueous extract of *H. italicum*

TEM images reveal that AgNPs are nearly spherical in shape with particle sizes ranging from 20 to 40 nm (Figure 2). The images also show that small particle aggregates are coated with a thin organic layer acting as a stabilizing agent. This explains the excellent dispersion of nanoparticles even at a macroscopic scale in the bio-reduced aqueous solution [21].

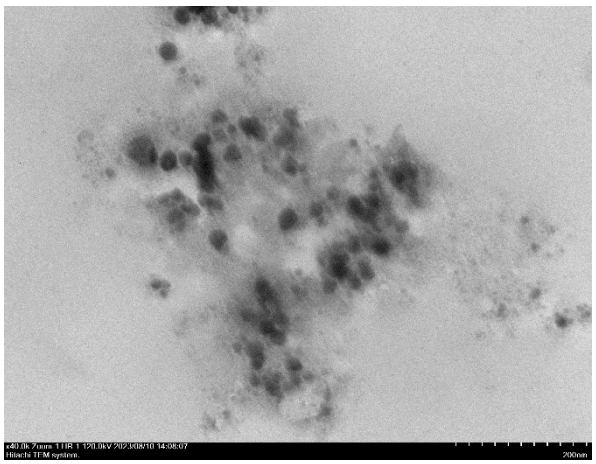

Figure 2 TEM images of AgNP

Mahapatra et al. [22] used creeping woodsorrel (*Oxalis corniculata*) to synthesize silver nanoparticles and examined their distribution and size using TEM analysis. They reported that their synthesized nanoparticles were approximately 40 nm in size and exhibited a spherical structure. Yassin et al. [23] analyzed the size and morphology of silver nanoparticles synthesized using marjoram (*Origanum majorana*) with TEM, reporting average diameter sizes ranging from 10 to 60 nm [24].

The morphology of AgNPs was determined through SEM characterization analyses. SEM images of the AgNP sample are presented in Figures 3a-b. Based on the obtained images, the nanoparticles synthesized in this study were found to be approximately 200 nm in size. Morphological evaluations revealed that the nanoparticles predominantly exhibited spherical and cubic structures. The data obtained in this study were consistent with findings reported in the literature [25].

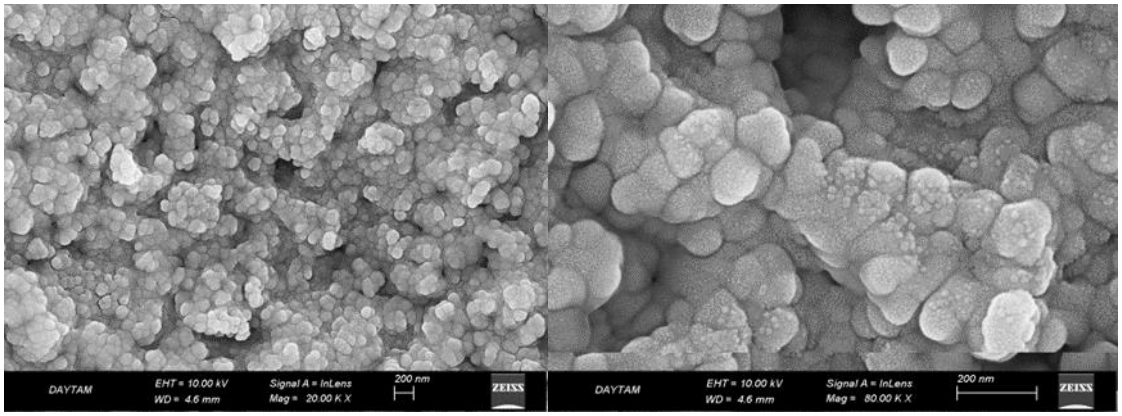

Figure 3a SEM images of AgNP (20.000X); b: SEM images of AgNP (60.000X)

One of the methods used to determine the elemental compositions of solid materials is EDS analysis [26]. In EDS analysis, silver ions produce strong signals around approximately 3.15 keV with occasional peaks, serving as evidence for the formation of silver nanoparticles [27]. The intermittent peaks typically characterized in our synthesized AgNPs are shown in the range of 0-3 keV. The presence of other weak elemental signals may be attributed to enzymes or proteins present in the *H. italicum* extract. The detection of elemental silver using EDS analysis is depicted in Figure 4.

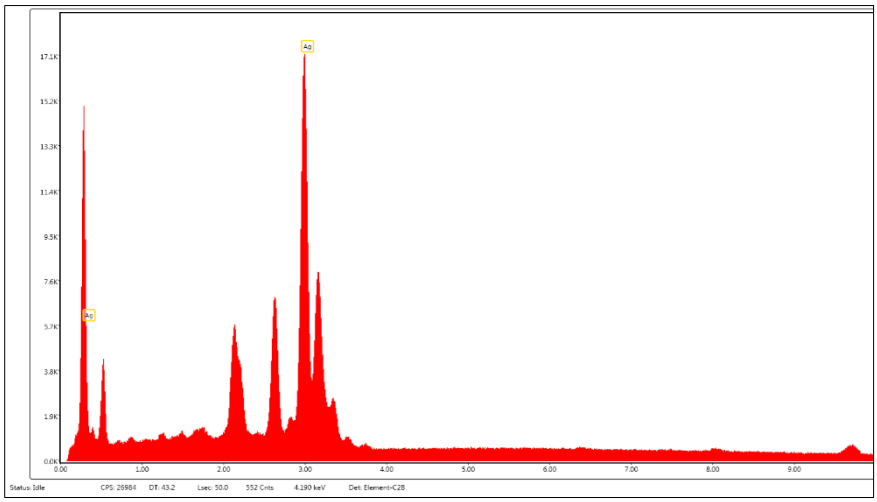

Figure 4 EDS images of AgNP

The maximum peak in the FT-IR spectrum was observed at 1550 cm^{-1}. These peaks are believed to be attributed to stretching and vibration between C-C aromatic bonds and C=O and OH groups. These peaks may arise from the characteristic properties of flavonoids/phenolic groups. Additionally, irregular small, wavy peaks were noted between 3400 - 3900 cm^{-1}, which are thought to result from stretching vibrations of O_2 and NH_2 (Figure 5). Zhang [28] analyzed the presence of functional groups of chemical compounds on synthesized AgNPs using FT-IR spectroscopy, where FT-IR spectra of AgNP samples exhibited absorption peaks between 3300 - 591 cm^{-1}.

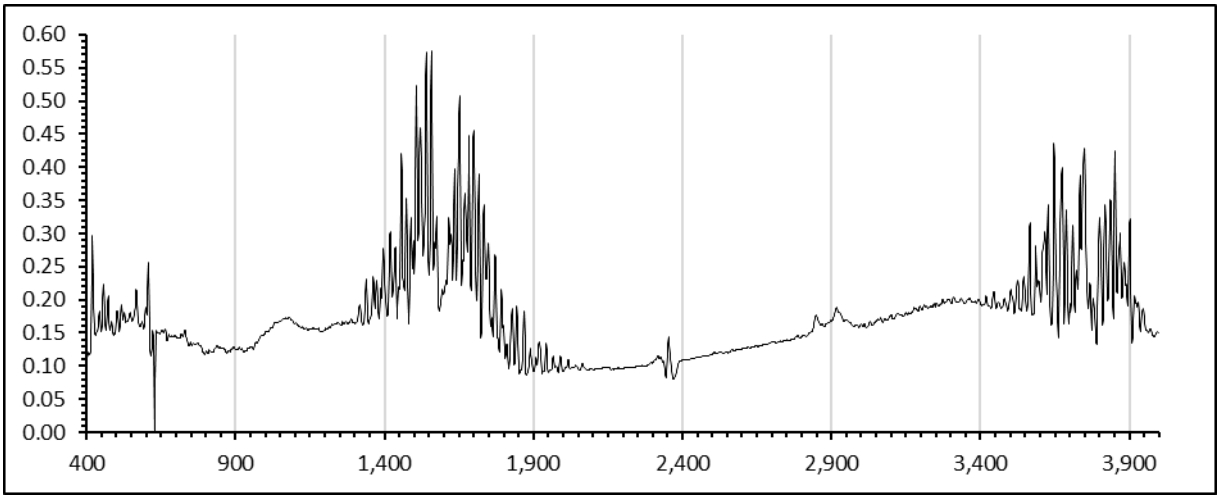

Figure 5 FT-IR images of AgNP

The peak at 431 nm in the UV-Vis spectrum originates from the plasmon resonance of metallic Ag particles (Figure 6). Vilas et al. [29] analyzed the formation of silver nanoparticles synthesized using the green synthesis method with *Coleus aromaticus* plant extract using UV-Vis spectroscopy. They determined that the AgNPs exhibited absorbance at 396 nm. Veisi et al. [30] synthesized spherical silver nanoparticles using *Citrus sinensis*, and confirmed nanoparticle synthesis with a peak observed at 412 nm in UV-Vis analysis. The UV-Vis spectroscopy analysis of the synthesized AgNPs in our study is consistent with literature findings [31].

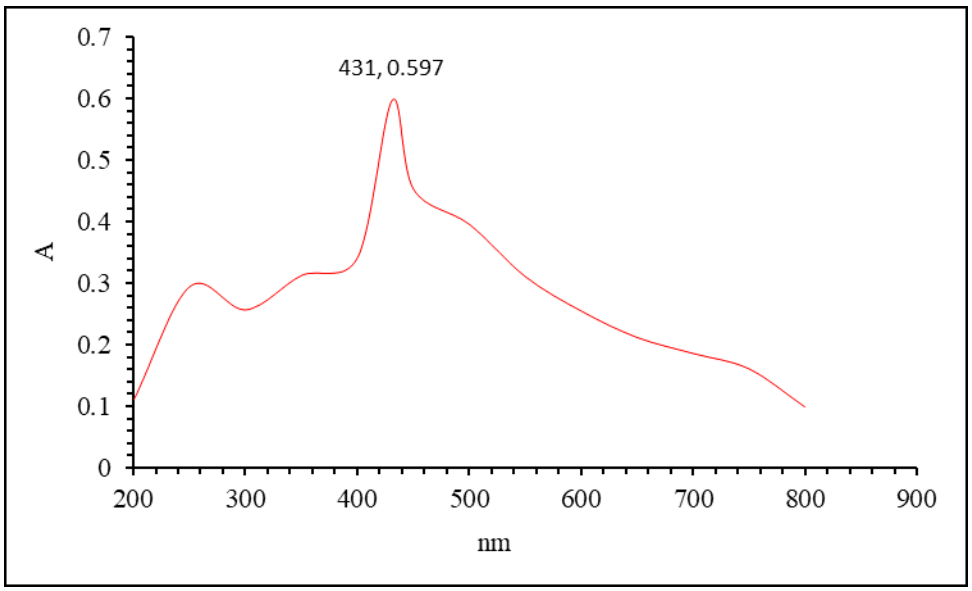

Figure 6 UV-Vis analysis of AgNP

In addition to AgNPs obtained by green synthesis, the antimicrobial activity of the extract obtained only from *H. italicum* was also examined to determine whether the antimicrobial effect was solely due to AgNPs (Table 1). In the study, disk diffusion method was used to measure zone diameters ranging from 7.00 to 21.00 mm for HIE and 7.00 to 30.00 mm for AgNP. The synthesized AgNP exhibited significantly higher antimicrobial activity against microorganisms other than *P. aeruginosa* ATCC 27853. The highest inhibition zone values were observed as 21.00 mm for HIE against *P. vulgaris* ATCC 13315, and 30.00 mm for AgNP against the same strain. The lowest inhibition zone values were 7.00 mm for HIE against *C. albicans* ATCC 10231 yeast culture, and also 7.00 mm for AgNP against *P. aeruginosa* ATCC 27853 bacteria. Compared to the control antibiotics used in the study, both HIE and nanoparticles showed larger inhibition zones against *S. aureus* ATCC 6538P, *E. coli* NRRL B-3704, *A. baumanii* ATCC 19606, and *P. vulgaris* ATCC 13315 bacteria.

Table 1 Antimicrobial activities of *H. italicum* and AgNP extracts

Test cultures	Methods									
	Disc Diffusion (mm)				MIC (µg mL^{-1})				MBC (µg mL^{-1})	
	Extracts		Control		Extracts (µg mL^{-1})		Control		Extracts (µg mL^{-1})	
	HIE	AgNP	P10	NY100	HIE	AgNP	S10	NY100	HIE	AgNP
B. subtilis ATCC 6633	11.00	12.00	14.00	NT	62.5	31.25	4.0	NT	125	62.5
S. aureus ATCC 6538P	20.00	16.00	13.00	NT	3.906	15.625	1.0	NT	7.812	31.25
S. haemolyticus ATCC 43252	15.00	10.00	16.00	NT	250	125	4.0	NT	500	250
E. coli NRRL B-3704	13.00	13.00	12.00	NT	125	62.5	2.0	NT	250	125
A. baumanii ATCC 19606	15.00	14.00	8.00	NT	500	62.5	4.0	NT	500	250
P. aeruginosa ATCC 27853	11.00	7.00	14.00	NT	62.5	31.25	4.0	NT	125	125
P. vulgaris ATCC 13315	21.00	30.00	15.00	NT	62.5	7.812	50	NT	62.5	7.812
C.albicans ATCC 10231	7.00	13.00	NT	16,00	500	125	NT	2.5	500	250

HIE: *H. italicum* extract; P10: Penicillin (10 ug/disc); S10: Streptomycin (10 ug/disc); NY100: Nystatin (100 ug/disc), Nt: not tested

MIC values were determined as 3.906 µg/mL for *S. aureus* ATCC 6538P with plant extract, and 7.812 µg/ml for *P. vulgaris* ATCC 13315 with AgNPs. Zakeri et al. [32] analyzed the antimicrobial activity of $AgNO_3$ solution using the disk diffusion method, obtaining inhibition zone diameters of 8.70 mm for *S. aureus* and 8.90 mm for *E. coli* bacteria. Sheikholeslami et al. [33] aimed to determine the MIC of AgNPs against *S. aureus*, *Staphylococcus epidermidis*, and *P. aeruginosa* bacteria. Their analysis showed MIC values of 62.5 µg/mL against *S. aureus* and 15.625 µg/mL against *P. aeruginosa*. Comparing these findings with the literature [18, 21, 24] both the plant extract and nanoparticles exhibited higher antagonistic activity against the test microorganisms in this study.

4. Conclusion

Typically, chemical methods used for NP synthesis involve environmental and biological risks associated with reducing agents and toxic organic solvents. Therefore, the use of plant extracts in NP synthesis is preferred as a more environmentally friendly approach, noted for being rapid, clean, non-toxic, and eco-friendly. In this study, *H. italicum* was chosen for NP synthesis due to its rich natural content of polyphenolic compounds and flavonoids. This work demonstrates for the first time that *H. italicum* extract can be used as a reducing agent for size- and shape-controlled NP synthesis, presenting a valuable resource for the biological synthesis of NPs in an environmentally sustainable manner. The use of *H. italicum* leaves as both reducing and stabilizing agents was highlighted in this study. This procedure offers a cost-effective and "green" alternative to traditional protocols, showcasing a synthesis process easily scalable for industrial applications, given its low synthesis temperatures and time requirements. However, the high biological activity results obtained suggest that optimization studies of the synthesis steps are necessary for further modification. In conclusion, leveraging the potential of endemic plants in our country to synthesize nanoparticles with different plant species represents a forward-looking goal.

Compliance with ethical standards

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Disclosure of conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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