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## Synthesis, characterization, and evaluation of the antimicrobial activities of silver nanoparticles from *Cyclotrichium organifolium* L.

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### Abstract

*Cyclotrichium organifolium* is a plant belonging to the *Lamiaceae* family and is a species that grows in the Western and Southern Anatolian regions of Turkey. In our study, the antimicrobial activities of silver nanoparticles (AgNP) were investigated through *Cyclotrichium organifolium* plant extract. Characterization processes of the obtained AgNPs, suitable spectral analysis methods; Uv-Vis was determined by FT-IR, SEM-EDX, XRD. According to the results of the analysis, it was determined that the AgNPs were spherical in shape and had an average diameter of 17.60 nm. The antimicrobial effect of AgNPs was determined by the minimum inhibition concentration (MIC) method. Gram positive as test microorganisms; *Staphylococcus aureus*, *Bacillus subtilis* and gram negative; *Escherichia coli*, *Pseudomonas aeruginosa* bacteria, and *Candida albicans* fungal pathogen species were used. The suppression of microorganism growth was investigated by comparing the efficacy of standard antibiotics used in our study with AgNPs produced by the green synthesis method. It has been observed that the obtained AgNPs have a very strong effect on gram-positive *B. subtilis* and gram-negative *E. coli* bacteria, and are more effective against *C. albicans* than the normal antifungal drug. It was determined that the antimicrobial activity of AgNPs produced from *C. organifolium* L. plants showed a stronger effect than standard antibiotics.

### Keywords

Antimicrobial, Nanoparticle, *Cyclotrichium organifolium* L., Silver nanoparticle, FESEM and TEM

### Introduction

Phyto-nanobiotechnology, which has made spectacular advancements in recent years and aims to synthesize nanoparticles using plant extracts, attracts the attention of scientists as a popular research area. Studies in this field is called as 'green synthesis.' This method, which is utilized as an alternative to physical and chemical synthesis, drives researchers to this field as it is more effective, simple to apply, non-harmful to human health and the environment, eliminates the waste product problem and is cost-efficient (Baran, 2019). Nanoparticles produced by the green synthesis method are employed as antiviral, antimicrobial, antioxidant, cytotoxic, antitumor,

anti-inflammatory, and bioremediation agents in the food and textile industries, smart agriculture, and wastewater treatment, today (Salem & Fouda, 2021).

The major advantage of using plant extracts in this method known as green synthesis is that they have a wide variety of metabolites which are readily available, safe, and non-toxic in most cases and can assist in the reduction of silver ions. In general, the therapeutic properties of plant products are associated with specific secondary metabolites such as tannins, saponins, flavonoids and alkaloids, and the reduction and stabilization of silver ions also occurs through these biomolecules (Ahmed et al.,

2019). Phytonanoparticles may function as carrier structures, distributing to cells for a variety of purposes. Their use in medicine and pharmacy has led to the development of novel approaches for drug production, diagnostic and treatment methods. These properties have made phytonanoparticles important tools at the molecular level (Ishak et al., 2019).

As long as pathogenic microorganisms gradually develop multi-antibiotic resistance, this makes it harder to access sustainable health services under economic circumstances and provides a necessity for novel and effective antimicrobial agents. Due to their properties, AgNPs produced by plants will lessen the side effects of drugs used in treatment while ensuring that they are more effective at lower dosages (Mohammed, 2015).

Infectious diseases and the associated mortalities have recently risen. One of the causes is that bacteria have begun to develop resistance to existing antibiotics, which they are genetically transmitting to new members. Therefore, more and combined antibiotics are required. Sometimes these medicine combinations are insufficient. Novel antimicrobial medications are required for this (Ranjbar et al., 2021). Plant bioactive components are one of the most plausible ways for novel antibiotics. Plants, in fact, are the earliest sources of antimicrobials that mankind has employed to fight diseases throughout history (Cowan, 1999).

*Cyclotrichium oranifolium* L. is one of the medicinal and aromatic plant belonging to the Labiatae family that grows mostly along the Mediterranean coasts. It is utilized locally as a medicinal herb (Kaya et al., 2000). In our study, the production of silver nanoparticles (AgNPs) by the green synthesis method by using *Cyclotrichium oranifolium* L. plant called mountain mint, collected from Garip village, Gelincik Mountain-Kızlar Pınarı village, Senirkent district, Isparta province, the determination of the characterization of these nanoparticles by various analyses, and their resistance against pathogenic microorganisms. It was aimed at evaluating its antimicrobial activity.

#### Material and Method

##### Collection of Plant Sample

The green leaves of the *Cyclotrichium oranifolium* L. (herba sideritis) plant used in the study were collected from its natural habitat in Isparta province, Senirkent district, Garip village, Gelincik Mountain-Kızlar Pınarı location (1500 m.) in June. The green leaves of *Cyclotrichium oranifolium* L. were first washed with tap water to remove dust and other residues. It was then washed three more times with distilled water. It was allowed to dry at room temperature ( $24 \pm 2$  °C) before being utilized in experimental studies.

##### Preparation of Plant Extract

The leaves of herba sideritis, cleaned and dried, were ground and weighed 50 g. It was allowed to boil for 2 hours at 85° with 500 ml of distilled water. The resultant extract was filtered using Whatman No.1 filter paper at room temperature (Ghosh et al., 2008). The obtained extract was stored at +4 °C.

##### Preparation of Silver Nitrate (AgNO<sub>3</sub>) Solution

Using alpha-aesier® AgNO<sub>3</sub> at an analytical purity of 99.8 %, a 1mM silver nitrate solution was prepared.

##### Production of Silver Nanoparticles

For the synthesis of silver nanoparticles, a 1mM AgNO<sub>3</sub> solution was utilized. 125 ml of plant leaf extract and 500 ml of AgNO<sub>3</sub> solution were mixed in a 2000 ml beaker at room temperature for one hour and allowed to react. Formation of AgNP was initially followed by the macroscopic method depending on the color change, and then the formation of nanoparticles was determined by scanning the wavelength through spectrophotometric measurements (Pugazhendhi et al., 2018). The resultant dark solution was centrifuged for 10 minutes at 7500 rpm. The liquid part that had accumulated on top was removed, and the remaining solid part was washed seven times with distilled water. The resultant AgNPs were dried in a drying oven at 65°C for 24 hours. The dry part (AgNPs) was ground with a glass stirrer and stored in a dark environment for characterization processes.

##### Characterization of Silver Nanoparticles

In order to determine the properties of AgNPs synthesized by biological method, (UV-1601 220V SHIMADZU) was utilized for analysis of ultraviolet-visible spectroscopy (Uv-vis), computer-controlled RadB-DMAX II for X-ray diffractometry (XRD) analysis, EVO 40 LEQ for Scanning Electron microscopy (SEM-EDAX) analysis, and Perkin Elmer Spectrum One® device for Fourier Transform Infrared spectroscopy (FT-IR) analysis. Characterization analyses were done as service procurement at the Inonu University Scientific and Technological Research Center (İBTAM).

##### UV-vis spectroscopic analysis of AgNPs formation

The color change of formed nanoparticles due to surface plasmon resonance and the formation of a characteristic absorption band at nanoparticle-specific wavelength is the first finding demonstrating nanoparticle formation (Paul et al., 2018). The formation of silver nanoparticles was monitored by color change. The ultraviolet spectra of the produced AgNPs were then determined using a 350-800 wavelength range spectrophotometer (UV-1601 220V Shimadzu®) (Baran et al., 2021).

##### Identification of the Crystal Structure of AgNPs

The crystal structures (XRD) of the obtained AgNPs were analyzed with RadB-DMAX II computer-controlled X-ray diffractometer in the range of  $1^\circ \leq 2\theta \leq 80^\circ$ . The average crystal particle sizes of the nanoparticles were assessed using the Debye-Scherrer equation during the XRD analysis.

##### Identification of AgNPs and Assessment of Their Shapes

The EVO 40 LEQ scanning electron microscope (SEM) was used to measure the morphology and size of the synthesized AgNPs. X-ray (EDX) spectroscopy was used to confirm the presence of AgNPs in the elemental composition and to determine its ratio.

##### FT-IR Analyses of AgNPs

FT-IR analysis in the 4000-400 cm<sup>-1</sup> range was performed to determine which functional groups are involved in the reduction of the extract produced before the reaction and the AgNPs forming as a consequence of the reaction.

##### Findings and Discussion

##### Analysis of UV-vis Spectroscopy

A dark brown color was quickly noticed, suggesting the formation of AgNP. The reaction extract and a 10 mM silver nitrate solution were mixed at 1:1 ratio to identify

the presence of AgNPs. Wavelength scanning was done on samples collected at 15, 30, 45, 60, 75, 90, and 120 minutes by a 1/10 dilution in the UV-vis. The maximum plasmon resonance of the formed AgNPs was found to be approximately 403 nm. In another study, Umaz et al.

reported the maximum plasmon resonance of AgNPs obtained from *Hypericum triquetrifolium Turra* plant extract as 453.91 nm (Adil et al., 2019). Figure 1 shows the UV-vis spectra of AgNPs depending on surface plasmon resonances (SPR).

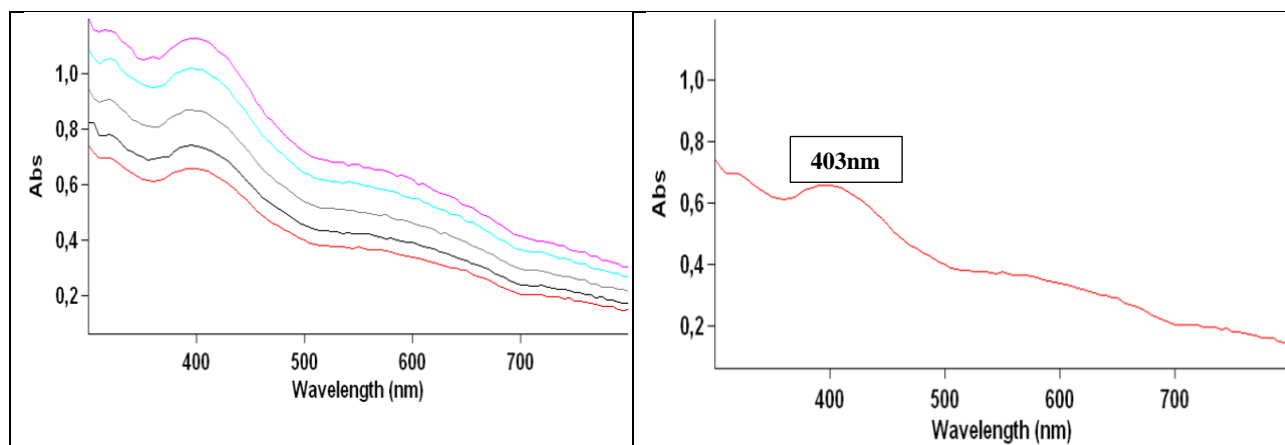


Figure 1. Time-dependent formation of AgNPs and the maximum absorbance value of AgNPs in UV-vis spectroscopy

### Fourier Transform Infrared Spectroscopy (FT-IR) Findings

The various functional groups involved in the reduction and stabilization of silver nanoparticles were identified using FT-IR analyses. The plant extract *Cyclotrichium organifolium* L. and the change in functional groups following synthesis were compared. The changes in peak values indicated that the functional groups were combined with silver. Considering the functional groups involved in nanoparticle formation, it can be asserted that the peaks at  $3332\text{ cm}^{-1}$  belonged to the

-OH group, the peaks at  $2145\text{ cm}^{-1}$  belonged to the  $\text{-C}\equiv\text{N}$  group and the peak at  $1635\text{ cm}^{-1}$  belonged to the  $\text{-C=O}$  (carbonyl) group. In similar study, the -OH,  $\text{C}\equiv\text{C}$ , and C-N functional groups took part in the reduction of nanoparticles derived from green olive leaves (*Olea europaea*) (Baran, 2019). Figures 2 and 3 show the FT-IR spectra of the leaf extract of *Cyclotrichium organifolium* L. plant.

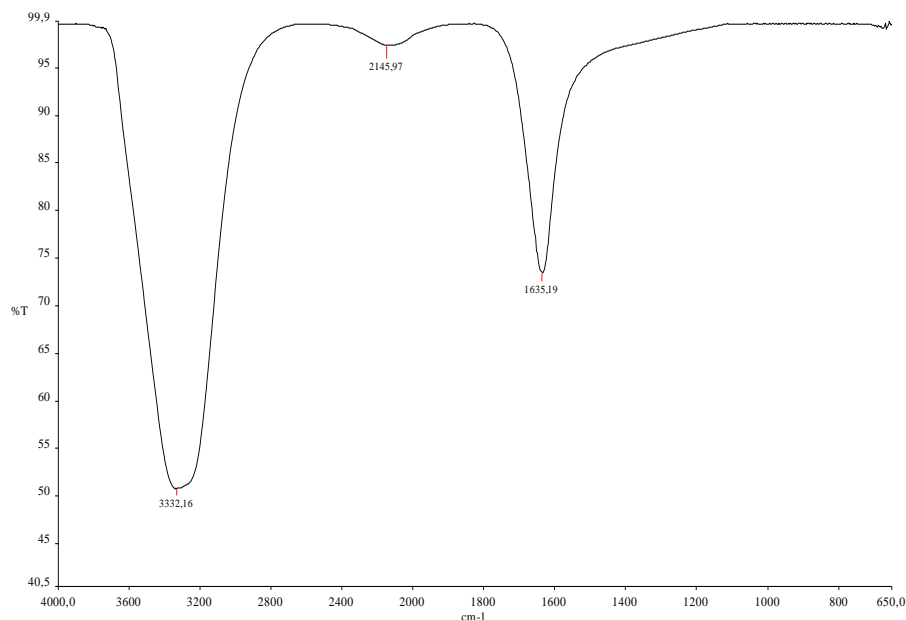


Figure 2. Assessment of functional groups involved in reduction before synthesis with FT-IR analysis

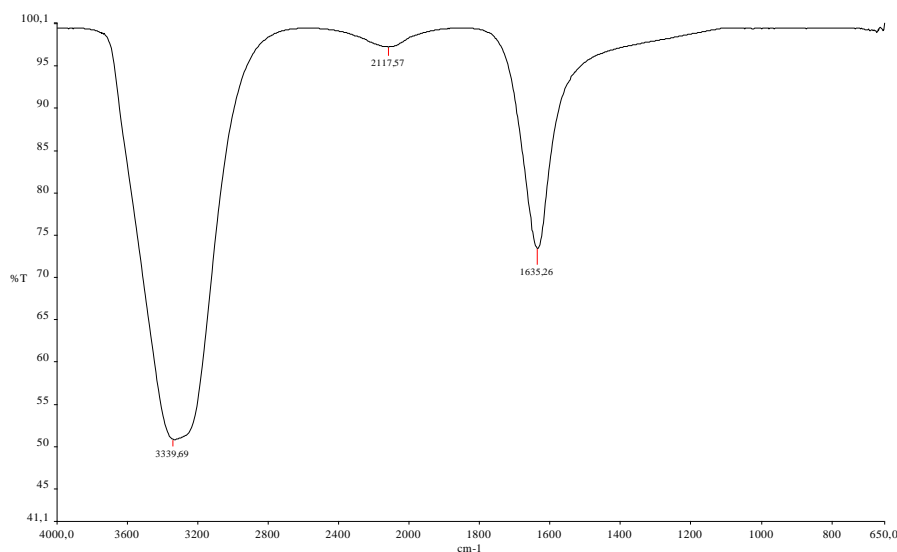


Figure 3. Assessment of functional groups involved in reduction after synthesis with FT-IR analysis

#### Size and Shape Analysis of AgNPs (SEM-EDX)

SEM analysis provides information on the sizes and morphological properties of the synthesized nanoparticles (Tripathi & Pandey-Rai, 2021). SEM was used to examine the surface morphology of nanoparticles produced from

the *Cyclotrichium organifolium* L. plant. When the analysis data were examined, the nanoparticles were observed to be spherical in shape with an average diameter of 17.60 nm (Figure 4).

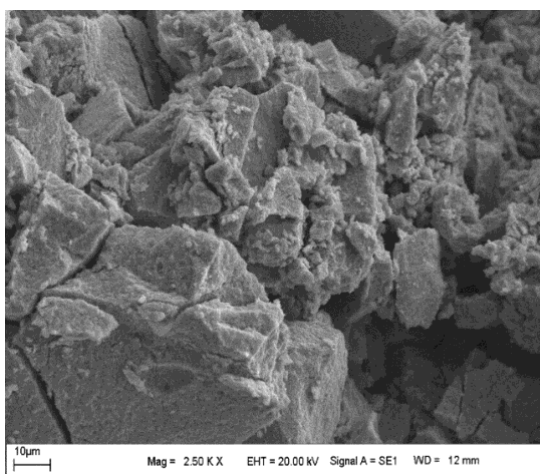
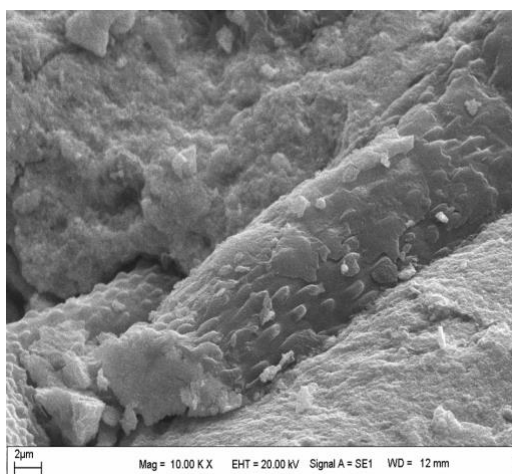


Figure 4. SEM images of silver nanoparticles produced from the leaf extract of the plant *Cyclotrichium organifolium* L.

The presence of silver was confirmed in the produced AgNPs using EDX spectroscopy (Baran et al., 2016); Aktepe and Baran 2021a). EDX analysis showed that the silver nanomaterial we produced was in elemental structure. The silver peaks in the diagram indicated that

the produced nanomaterial contained considerably silver (Figure 5). Other signals observed, other than silver, might have come from other biomolecules around AgNPs (Tripathi & Pandey-Rai, 2021).

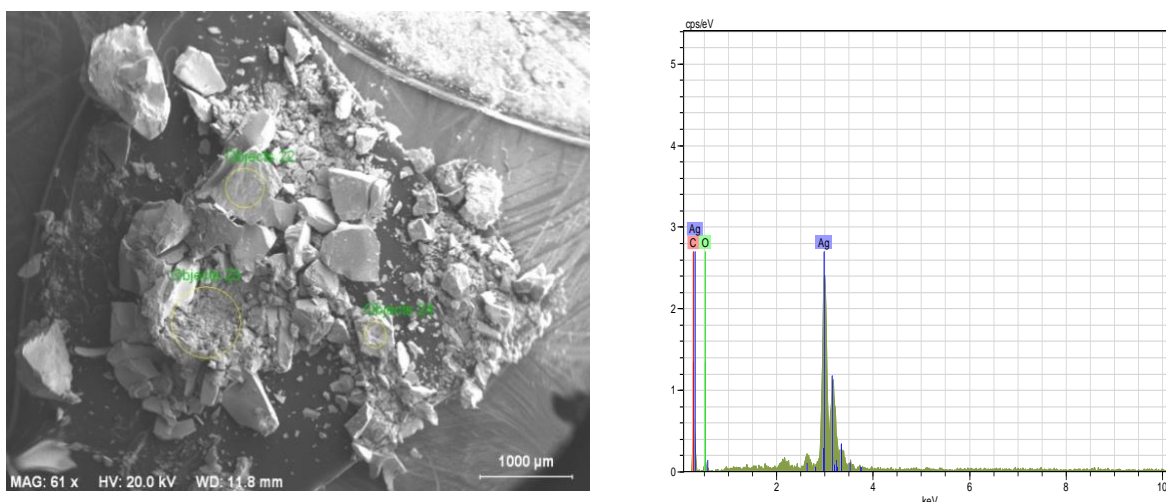


Figure 5. Assessment of elemental compositions by EDX analysis of AgNPs

### Crystal Structure Analysis (XRD) of AgNPs

The X-ray Diffraction technique (XRD) is based on the method by which a crystal refracts X-rays in different directions depending on its unique atomic and molecular arrangement. The diffraction profiles acquired for each crystal phase are surrounded by spot patterns. For each crystal, these patterns are as unique as fingerprints (Şahin, 2019); Acay and Baran 2019; Acay, et al., 2019).

XRD analyses were done on AgNPs synthesized by biological method from the *Cyclotrichium origanifolium* L. plant. The peaks at  $111^\circ$ ,  $200^\circ$ ,  $220^\circ$ , and  $311^\circ$

corresponding to  $2\theta$  demonstrate the spherical crystal structure of silver, as shown in Figure 6. The crystal size of AgNPs was calculated using the values corresponding to these peaks ( $38.11^\circ$ ,  $44.30^\circ$ ,  $64.44^\circ$ , and  $77.40^\circ$ ). Using the Debye-Scherrer formula, the size of AgNPs was found to be around 17.60 nm. It was calculated using the inequality  $D = K\lambda/(\beta \cos\theta)$  (Abdullah & Baran, 2019).

Where  $D$  = the particle size (nm),  $K$  = constant (0.90),  $\lambda$  = X-ray wavelength ( $1.5406^\circ\text{Å}$ ),  $\beta$  = half the value of the highest peak (FWHM),  $\theta$  = angle of refraction.

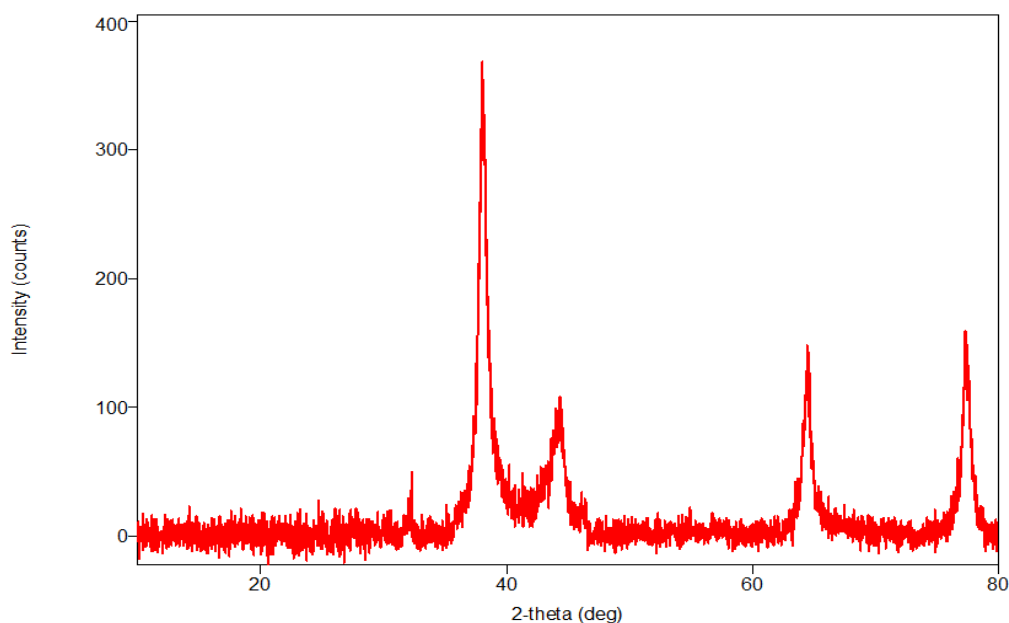


Figure 6. XRD Analysis data of AgNPs

When the XRD analysis of the synthesized AgNPs was examined, four intense peaks is seen in Figure 6 in the spectra of  $2\theta$  values between  $30^\circ$  and  $80^\circ$ .

### Measurment of Antimicrobial Activity

Pathogenic gram-positive *Staphylococcus aureus* (ATCC 29213), *Bacillus subtilis* (ATCC 11774), gram-negative *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) strains, and *Candida albicans* (ATCC 10231) yeast were employed in

antimicrobial activity studies. The microorganisms employed in the study were obtained from the laboratory of the Mardin Artuklu University Health Sciences Vocational School. The antimicrobial effects of silver nanoparticles produced by the green synthesis method were investigated using the microdilution method depending on the determination of minimum inhibitory concentration (MIC) values.



In the microplate wells, Muller Hinton medium, AgNPs produced from the plant extract at different concentrations, and a mixture of microorganisms prepared according to McFarland standard 0.5 (turbidity) were placed. Commercially available standard antibiotics vancomycin for gram-positive bacteria, colistin for gram-negative bacteria, and fluconazole for *C. albicans* yeast were used to compare the antimicrobial effects of plant AgNPs. The antimicrobial effects of AgNPs at 1 mM concentration on microorganisms were examined in this study. To that end, 100 µL of AgNP solution obtained from 1 mg/mL plant extract was added to the first well. After pipetting, 100 µL of liquid was drawn from the first well and added to the second well. After repeating the

procedure until the tenth well, 100 µL of liquid was drawn and thrown out. Thus, in each well, 100 µL of medium and McFarland solution containing 100 µL of bacteria remained. As a result, the plant extract with a baseline concentration of 1 mg/mL was half in concentration after each transfer.

AgNPs produced from the *Cyclotrichium origanifolium* L. plant by green synthesis method were compared based on 1 mM silver nitrate (AgNO<sub>3</sub>) and MIC (minimum inhibitory concentration) values of commercial antibiotics. The antimicrobial activity of AgNPs was found to be more effective than the antibiotics used and 1 mM AgNO<sub>3</sub> solution (Table 2).

Table 1. Antimicrobial effect of AgNPs against gram-positive and gram-negative pathogenic bacteria strains, as well as *Candida albicans* yeast

	Tested microorganisms	AgNPs mg/L	AgNO <sub>3</sub> Solution mg/L	Antibiyotik mg/L
Gram (+) bacteria	<i>S. aureus</i> ATCC 29213	1.0	2.65	2
	<i>B. subtilis</i> ATCC 11774	0.05	1.32	1
Gram (-) bacteria	<i>E. coli</i> ATCC 25922	0.10	0.66	2
	<i>P. aeruginosa</i> ATCC 27853	0.50	0.66	2
Yeast	<i>C. albicans</i> ATCC 10231	0.50	0.66	2

In the present study, AgNPs were found to be to be effective against gram-positive bacteria pathogenic to humans, *S. aureus* (ATCC 29213) and *B. subtilis* (ATCC 11774), at concentrations of 1.0 and 0.05 mg/mL, respectively. It was also demonstrated that it had a more antifungal effect on *C. albicans* fungus than classical antifungals and silver nitrate compounds. For *S. aureus*, produced AgNPs were twice as effective as the antibiotic and two and a half times more effective than silver nitrate solution. When the MIC values for *B. subtilis* bacteria were investigated, AgNPs were observed to be twenty times more effective than antibiotics and approximately twenty-five times more effective than silver nitrate solution. According to these findings, gram-positive *B. subtilis* bacteria were more susceptible to AgNPs than *S. aureus* bacteria. In a study, AgNPs produced from the *Sida cordifolia* plant were reported to be effective on *B. subtilis* bacteria at the concentration of 6.25 mg/mL (Pallela et al., 2018). In another study, the MIC value of biological AgNPs produced from *Streptomyces xinghaiensis* OF1 strain for *S. aureus* was reported as 256 mg/mL (KESKİN & GÜVENSEN, 2022; Wypij et al., 2018).

The MIC values of AgNPs produced for Gram-negative *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) bacteria were found to be 0.10 and 0.50 mg/mL, respectively. When the data in Table 1 were examined, it was understood that AgNPs were more effective than both silver nitrate and the antibiotics utilized. In a previous study, the MIC values of AgNPs produced from the fruit extracts of *Crataegus pentagyna* plant against *E. coli* and *P. aeruginosa* bacteria were found to be 0.11 - 0.22

mg/mL, respectively (Ebrahimzadeh et al., 2020). The MIC value of AgNPs produced from *Zea mays* L. plant leaves on *E. coli* bacteria was measured to be 0.084 in another study (Baran, 2019). The MIC of AgNPs produced from the medicinal plant *Holarrhena pubescens* against imipenem-resistant clinical isolates of *P. aeruginosa* was reported to be 20 mg/mL (Ali et al., 2018).

The MIC value of AgNPs utilized against the pathogenic *C. albicans* (ATCC 10231) yeast, which causes most frequently the infection in humans, was measured to be 0.50 mg/mL. This value indicates that it is four times as effective as the fluconazole antifungal medicine used as a positive control. AgNPs were also determined to be more effective than the silver nitrate solution utilized in the study (Table 1). The MIC value of AgNPs produced from the plant extract of *Hypericum triquetrifolium* Turra against *Candida albicans* yeast was measured to be 0.02 mg/mL in a study investigating the antifungal effects of AgNPs (Adil et al., 2019). AgNPs produced from the *Fusarium oxysporum* plant were found to have a fungicidal effect on *C. albicans* fungi resistant to fluconazole-type antibiotics, with MIC values ranging from 2.17 to 4.35 mg/mL (BARAN & YEŞİLADA, 2022; Longhi et al., 2015). In another study, the antifungal activity of AgNPs produced from iturin, a cyclic peptide with known antifungal activity, was tested in vitro against *C. albicans*. AgNPs produced from iturin have been reported to be highly efficient at MIC values ranging from 1.25 to 2.5 mg/mL (Zhou et al., 2021). The fungicidal effects of AgNPs produced from *Artemisia annua* and *Curcuma longa* plants by the green synthesis method

against *C. albicans* fungus were determined by measuring their MIC values (Paul et al., 2018). All of these studies reveal that AgNPs produced from the green synthesis method are effective against various microorganisms, and the MIC values of AgNPs produced from different sources against the same microorganism might be extremely different.

### Conclusion and Recommendations

Understanding that the by-products of nanoparticles synthesized by physical and chemical methods have major toxic impacts has driven studies in this field to biological synthesis methods that include safer, easier, affordable, and environmentally friendly applications.

Silver is a potentially toxic metal. Silver is one of the most researched metal nanoparticles due to its antimicrobial characteristics. Nanosilver, whose application area is continually growing, would inevitably meet with the production of pharmaceuticals with detailed studies and controlled experiments. The development of multi-drug resistance by some microorganisms results in the inability to treat microorganism-based diseases, posing major health consequences. Silver nanoparticles produced using the green synthesis method may be useful at this point. Numerous studies in this field now have revealed that AgNPs produced using the green synthesis method are resistant to microorganisms.

In the present study, targeted silver nanoparticles were successfully obtained from the leaves of *Cyclotrichium origanifolium* L. (herba sideritis) plant using a phyto-nanotechnological method, under mild and reproducible reaction conditions, with a practical method, and without using a chemical reducing agent in accordance with the principles of green synthesis. The structural characteristics, shapes, sizes, crystal structures, and element compositions of the silver nanoparticles we produced were examined using appropriate characterization methods (UV-vis, FT-IR, SEM-EDX, XRD), and the findings were found to be consistent with published data.

Today, there has been an increase in inflammatory, degenerative, cancerous, and infectious diseases. People develop resistance to the traditional medications over time, therefore, they are inadequate. Inadequate preventive and therapeutic methods for such diseases, as well as exposure to undesirable effects, increase the significance of treatment with natural active substances derived from herbs as opposed to synthetic drugs. In the present study, it was observed that AgNPs produced from plant extract were more effective against sample pathogenic microorganisms than antimicrobial medicines and yielded better outcomes. Nevertheless, number of these studies is limited. Further studies are needed in this field.

### 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

Ethics committee approval is not required.

#### Funding

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#### Data availability

Not applicable.

#### Consent for publication

Not applicable.

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