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Environmentally Friendly Rapid Synthesis of Gold Nanoparticles from *Artemisia absinthium* Plant Extract and Application of Antimicrobial Activities

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ABSTRACT: Nanoparticles are used in many areas due to their superior properties. Recently, producing these particles using environmentally friendly synthesis methods has become very popular. Within this context, in this study, gold nanoparticles (AuNPs) were synthesized by using a quick, eco-friendly method with the green leaf extract of *Artemisia absinthium* (wormwood). The grain size of the AuNPs was determined as 13.40 nm according to the Debye-Scherrer equation. The inhibitory effects of the AuNPs on the growth of the tested microorganisms were determined by the minimum inhibitory concentration (MIC) method. The MIC is described as the minimum concentration of an antimicrobial agent that inhibits the growth of a microorganism. The obtained AuNPs were showed inhibitory effects on *S. aureus*, *S. pyogenes*, E. coli, *P. aeruginosa* strains and *C. albicans* yeast at concentrations of 0.250, 0.125, 0.125, 0.033 and 0.66 mg mL^{-1} respectively.

Keywords: Antimicrobial, gold nanoparticles, green synthesis, SEM-EDX, TGA, XRD

Artemisia absinthium Bitki Ekstraktından Altın Nanopartiküllerin Çevre Dostu Hızlı Sentezi ve Antimikrobiyal Aktivitelerin Uygulanması

ÖZET: Nanopartiküller, üstün özelliklerinden dolayı birçok alanda kullanılmaktadır. Son zamanlarda, bu parçacıkların çevre dostu sentez yöntemleri kullanılarak üretilmesi çok popüler hale gelmiştir. Bu bağlamda, bu çalışmada, altın nanopartiküller (AuNP'ler), *Artemisia absinthium*'un (pelin otu) yeşil yaprak özütü ile hızlı ve çevre dostu bir yöntem kullanılarak sentezlenmiştir. AuNP'lerin tane boyutu Debye-Scherrer denklemine göre 13.40 nm olarak belirlendi. AuNP'lerin test edilen mikroorganizmaların büyümesi üzerindeki inhibe edici etkileri, minimum inhibe edici konsantrasyon (MİC) yöntemi ile belirlenmiştir. MİC, bir mikroorganizmanın büyümesini engelleyen bir antimikrobiyal maddenin minimum konsantrasyonu olarak tanımlanmaktadır. Elde edilen AuNP'ler, sırasıyla 0.250, 0.125, 0.125, 0.033 ve 0.66 mg mL^{-1} konsantrasyonlarında *S. aureus*, *S. pyogenes*, *E. coli*, *P. aeruginosa* suşları ve *C. albicans* mayası üzerinde önleyici etkiler göstermiştir.

Anahtar Kelimeler: Antimikrobiyal, altın nanopartiküller, yeşil sentez, SEM-EDX, TGA, XRD

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INTRODUCTION

Metal nanoparticles have superior chemical and physical properties (Huang and Yang, 2004). Due to these properties, they are widely used in many sectors such as the food, cosmetics, textile, optics and electronics, healthcare and biomedical (Khan et al., 2017; Chaudhry, 2018; Shao et al., 2018; Shaid et al.,2018; Santhosh et al., 2019; Shahid et al., 2019; Khan et al., 2020). Metal nanoparticles can be procured by using physical, chemical and biological methods. However, there are various disadvantages to using the physical and chemical methods such as the fact that they contain toxic chemicals in the synthesis stage, require high energy and are costly (Gunalan et al., 2012; Kumar et al., 2018; Kanchi et al., 2018; Jeyaraj et al., 2019). Thus, biological methods are more attractive compared to these two methods. Plants, bacteria, fungi and algae can be used in the biological synthesize of AuNPs (Dubey et al., 2010; Rajathi et al., 2014). The interest in plant-based synthesis methods increases by day because they are easy to operate and eco-friendly. Vegetable sources such as seeds, leaves, and fruit can be used as a source in AuNPs synthesis (Mythili et al., 2018). The phytochemicals in plant extracts produce AuNPs by reducing the gold metal salt in aqueous solutions (Aravinthan et al., 2016; Teimuri-mofrad et al., 2017).

AuNPs have led to significant improvements in biology and medicine (Ankamwar et al., 2010; Giljohann et al., 2010). Gold nanoparticles (AuNPs) are used in catalysis, anti-cancer agents, agriculture, the pharmaceutical industry, anti-microbial and anti-oxidant agents and many bacteria, algae, and fungi (Philip, 2010; Kumar et al., 2013; Rajathi et al., 2014; Balalakshmi et al., 2017; Menon et al., 2017; Kobashigawa et al., 2018). AuNPs are biocompatible and their unique surface plasmon resonance (SPR) peaks are important for biomedical uses (Patra et al., 2016; Murphin Kumar et al., 2017). *Zingiber officinale* (Menon et al., 2017), *Butea monosperma* (Balalakshmi et al., 2017), *Terminalia catappa* (Philip, 2010), *Mentha piperita* (Kumar et al., 2013), *Alcea rosea* (Khoshnamvand et al., 2019) just as, green synthesis of AuNPs was carried out from plant sources.

In this study, AuNPs were synthesized with the plant extract of *Artemisia absinthium* (Wormwood herb) by using an environmentally friendly, easy and cost-effective method and their effects on the reproduction of pathogenic microorganisms were investigated. The syntheses of the NPs with different experimental methods are schematized in Figure 1.

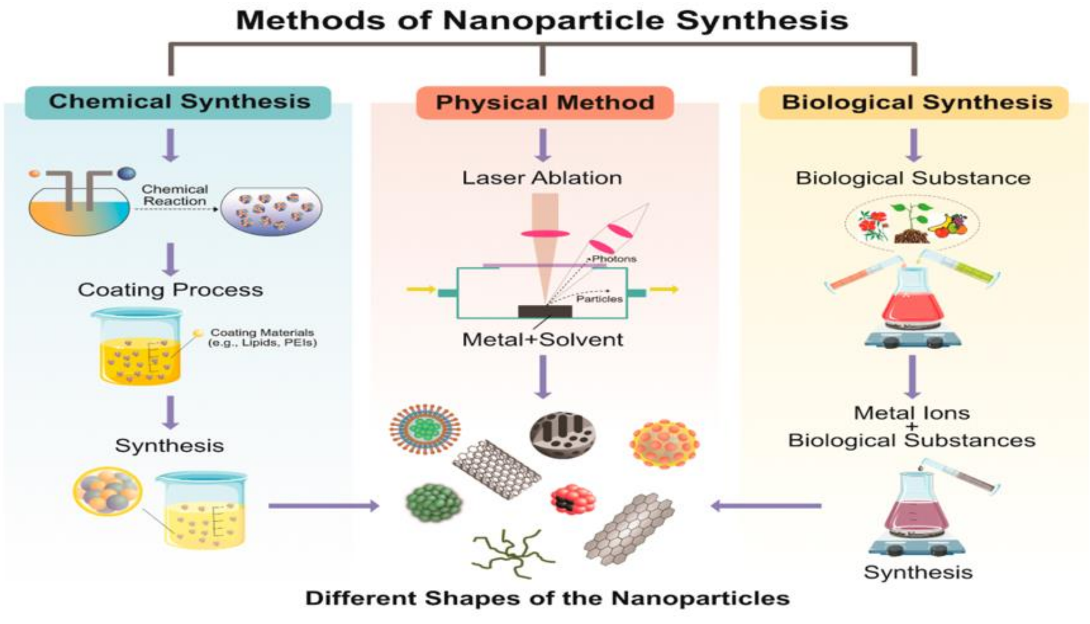

Figure 1. Schematic view of the commonly used nanoparticle synthesis methods (Jeyaraj et al., 2019)

MATERIALS AND METHODS

Materials

Preparation of the Wormwood Herb extract and gold tetrachloride ($HAuCI_4.3H_2O$) solution

The plant samples were collected in the Savur district of the province of Mardin during the summer. The samples were initially washed with tap water and then washed several times using distilled water. The leaves of the plant samples were left to dry for 48 hours in room conditions. A total of 50 g of the dried plant leaves were placed into a 500 ml beaker, then 250 ml of distilled water was added and the mixture was boiled. To create the desired reaction, the mixture was boiled for 5 minutes. The extract was then left to cool in room temperature. Filtering was carried out with coarse filter paper and Whatman filter paper No. 1. The obtained extract was stored at +4 $^{\circ}$C until the experiments were conducted. A 1 millimolar (mM) gold solution was produced for use in synthesis from the gold tetrachloride ($HAuCI_4.3H_2O$) of 49.0% purity, which was commercially purchased from Sigma-Aldrich. The Synthesis and experiment steps of the AuNPs are schematized in Figure 2.

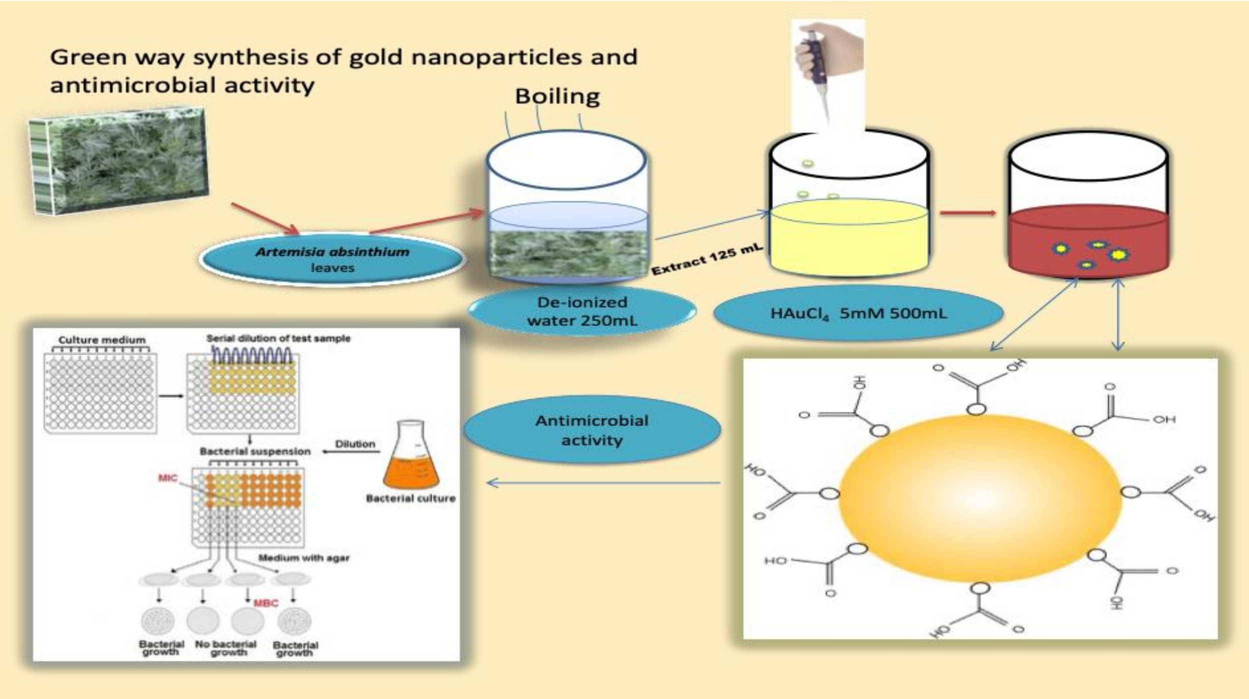

Figure 2. Schematic view of the synthesis of AuNPs with wormwood herb leaves extract.

Biosynthesis

Exactly 50 ml of the extract prepared from the leaves of the Wormwood herb and 250 ml of $HAuCI_4$ solution were placed in a 1000 ml flask and left to react at 45 $^{\circ}$C after being simply shaken by hand. The reaction mixture was found to change color in accordance with time. The liquid content obtained as a result of the reaction was centrifuged with an OHAUS FC 5706 device at 6.000 rpm for 15 minutes. After being washed several times, the precipitated solid was left to dry in an oven at 75 $^{\circ}$C for 24 hours. The obtained particles were then prepared for characterization.

The phytochemicals in plant extracts reduce Au^{+3} to Au^0 and, thus, form AuNPs (Patra et al., 2016; Murphin Kumar et al., 2017). Such extracts are both reducing and stabilizing (Giljohann et al., 2010).

Characterization

UV–visible spectroscopy

The absorbance values depending on physical color change (0, 5, 10, 20, 30, 40, 50, 60, 90, 120 and 180 minutes) were examined by using an Agilent Cary 60 UV-visible (UV-vis) spectrophotometer. UV-vis spectroscopy was used to observed the surface plasmon resonance (SPR) bands of the biosynthesized AuNPs. Thus, the formation and presence of the AuNPs were evaluated.

Fourier transform infrared spectroscopy

To evaluating the phytochemicals and functional groups responsible for synthesis (reduction reaction) in wormwood leaf extract used, the measurement was performed with Fourier transform infrared (FT-IR) spectroscopy (Perkin Elmer Spectrum One instrument).

Energy dispersive X-ray spectrum and scanning electron microscopy

The morphological images and element composition of the particles were evaluated with EVO 40 LEQ scanning electron microscopy (SEM) - energy dispersive X-Ray spectroscopy (EDX) device.

X-ray diffraction

The crystal structure of the particles was evaluated by a RadB-DMAX II computer-controlled X-ray diffractometer analysis, while the crystal particle size was calculated using the Debye-Scherer equation (Jeyaraj et al., 2019).

Thermal gravimetric analysis

Using a Shimadzu TGA-50 device, the degradation data of the AuNPs between 25-1000 $^{\circ}$C and the thermogravimetric analysis (TGA) of the particles with a flow rate of 20 mL/min in the atmosphere of N_2 (g) with a heating rate of 10 $^{\circ}$C /min, and their durability were checked after heat treatment.

Preparation of the Broth and Microorganism Suspensions for Antimicrobial Activity

Investigations were performed to determine the suppressing effects of the AuNPs on the growth of micro-organisms by the microdilution method to determine the minimum inhibitory concentration (MIC). Microorganism suspensions were prepared according to the 0.5 McFarland (McFarland, 1907; Gholami-Shabani et al., 2015) concentration with Gram-negative *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, gram-positive *Staphylococcus aureus* ATCC 29213, *Streptococcus pyogenes* ATTC 19615 bacteria and *Candida albicans* ATTC 10231 yeast.

A Muller Hinton liquid medium was used for the bacteria, while an RPMI medium was used for *C. albicans*. Media, nanoparticle and microorganism suspensions were added to the microplates and the inoculated cultures were incubated at 37 $^{\circ}$C for 24 h. The antimicrobial effects of the commercially available colistin, fluconazole, vancomycin antibiotics and 1 mM gold solution were also examined for comparison.

RESULTS AND DISCUSSION

UV–visible spectroscopy analysis

After the extract of the wormwood herb and 1 mM $HAuCl_4$ (gold solution) were mixed the color change was observed. Measurements were taken at different time intervals, namely, 0, 5, 10, 20, 30, 40, 50, 60, 90,120 and 180 minutes, using a UV-vis spectroscopy and the color was found to change from light yellow to dark pink-red with the formation of vibrations on the SPR (Dubey et al., 2010) due to reduction (Figure 3). In a study, in which the synthesis of AuNPs was performed using the extract of the *Salvia officinalis* plant, it was determined that the color change from yellow to dark pink was linked to the formation of the AuNPs (Mythili et al., 2018). A similar conclusion was also made in a study in which the biological synthesis of AuNPs was carried out with the leaf extract of *Ziziphus zizyphus* (Aravinthan et al., 2016). When the UV-vis spectra of the present study were examined, it was determined that the maximum absorbance value at 528 nm indicated the formation and presence of the AuNPs. In a biosynthesis study conducted with the leaf extract of *Satureja hortensis*, the absorbances of AuNPs at a maximum wavelength of 528 nm were found (Teimuri-mofrad et al., 2017). Absorbance band values in the range of 500–600 nm indicate the presence of AuNPs (Mubarakali et al., 2011). In a different synthesis study performed using the extract of the *Marsdenia tenacissima* plant, the absorbance peaks between 525-540 nm were linked to the formation of AuNPs (Khoshnamvand et al., 2019).

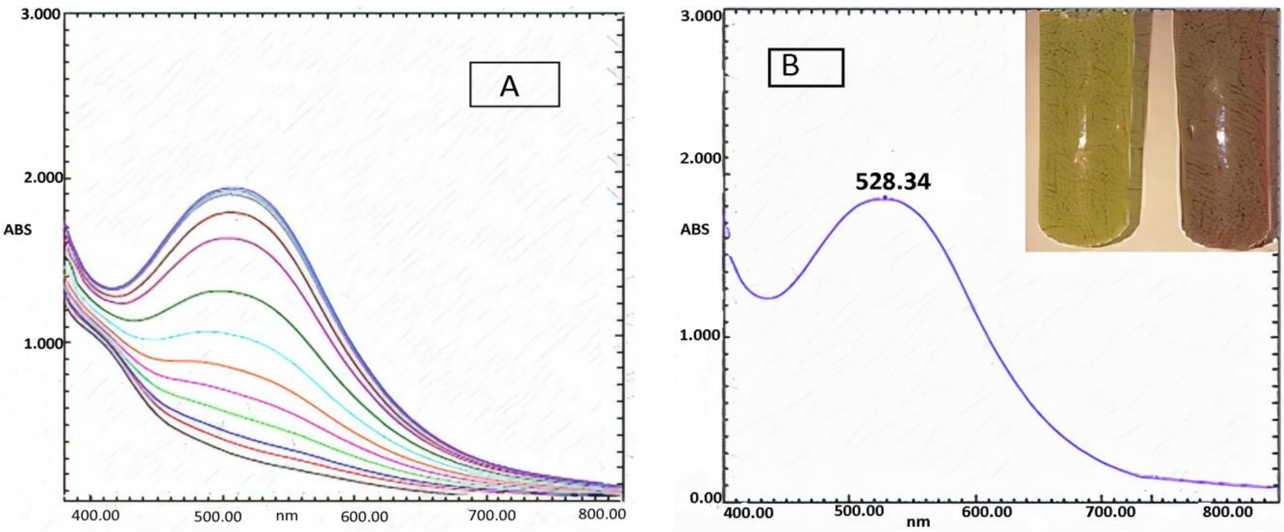

Figure 3 A. Result of UV -Vis spectroscopy analysis at the time-dependent formation of AuNPs. **B.** Maximum absorbance value of AuNPs by using UV-Vis Spectrophotometer

Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared **(**FT-IR) spectroscopy was used to characterize the functional groups responsible for the reduction of the phytochemicals in the Wormwood herb extract, which was used for synthesis. It can be said that the changes in the functional groups after the reduction in the FT-IR spectra were responsible for the formation of AuNPs by taking an active role in the reduction of the phytochemicals in the extract (Figure 4). When the functional groups that took part in the reduction during the formation of AuNPs were examined, it was observed that the peak between 3325.68-3324.10 cm^{-1} caused -OH stretching, the peak between 1636.31-1634.82 cm^{-1} caused –C = O I amide band and the peak at 2360 cm^{-1} caused aldehyde related -CH stretching. After the extract and metal salt interaction, it was determined that these groups may be responsible for the reduction of the 1-2 cm^{-1} change in the –OH and –C = O groups, respectively (Figure 4). Aldehyde groups are considered to be one of the functional groups responsible for the reduction in synthesis (Khoshnamvand et al., 2019). In herbal synthesis studies that supported the data of the present study, it was reported that the NH and/or OH, (C=O) NH_2 groups were found to be responsible for reduction (Dwivedi et al., 2010; Kumar et al., 2013).

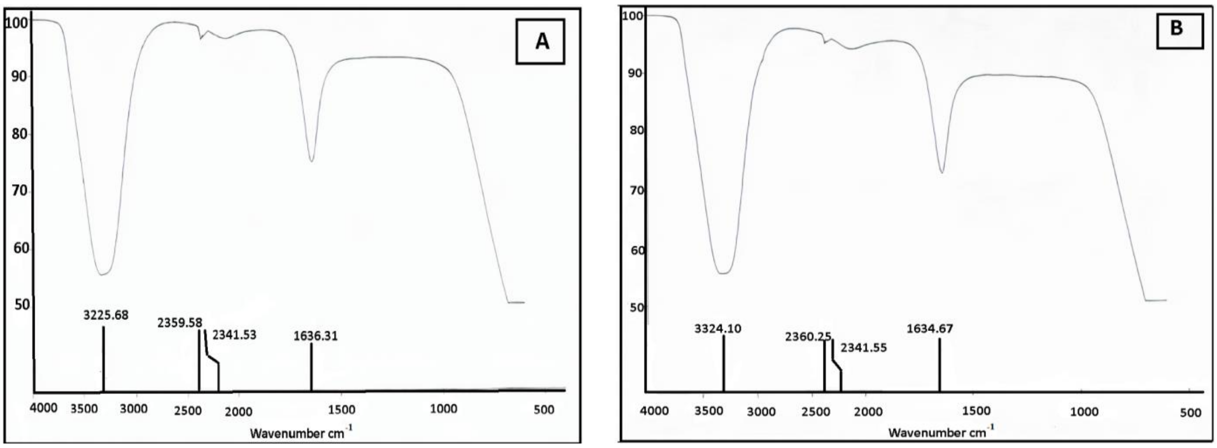

Figure 4 A. FTIR Analysis of obtained extract from the leaf of Wormwood herb **B.** Changes of existing functional groups in Wormwood herb after synthesis.

Energy dispersive X-ray spectrum and scanning electron microscopy analyses

Based on the EDX data, the element composition was found to be comprised mainly of gold. Other peaks were caused by pollution from the Wormwood herb extract (Figure 5). Similar results were obtained in synthesis studies conducted with *Cymbopogon citratus* and *Pistia stratiotes* extracts using an environmentally friendly method as the peaks corresponding to the gold in the composition of AuNPs were determined (Anuradha et al., 2015; Murugan et al., 2015). According to the SEM images, the AuNPs exhibited spherical and rectangular morphology below 100 nm. In a synthesis study conducted with *Cinnamomum camphora* extract, AuNPs were found to exhibit spherical appearance between 55-80 nm (Oueslati et al., 2020). AuNPs synthesized with *Trianthema decandra* L. and *Andrographis peniculata* extracts were reported to exhibit spherical morphology between 37-79 and 56±12 nm, respectively (Aljabali et al., 2018).

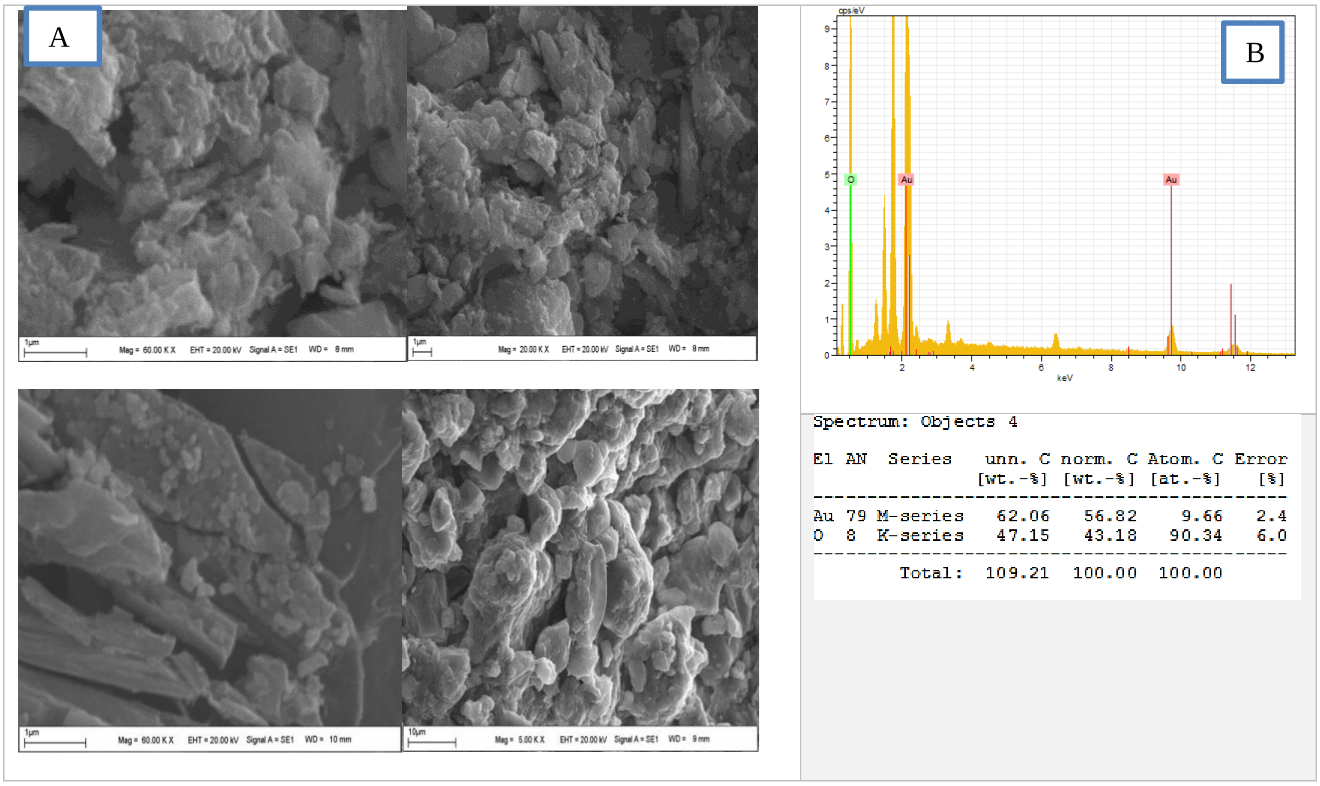

Figure 5 A. SEM results of AuNPs **B.** EDX profile of AuNPs.

X-ray diffraction analysis data

When X-ray diffraction (XRD) analysis data was examined (Figure 6), it was determined that the peaks at 111°, 200°, 220° and 311°corresponding to 2θ showed the cubic crystal structure of AuNPs. The numerical values corresponding to these peaks were 38.053, 44.24, 66.99 and 77.71 respectively. In the synthesis with the extracts of the *Satureja hortens* (Gharehyakheh et al., 2020), *Marsdenia tenacissima inhibits* (Sun et al., 2019) and *Ginkgo biloba* (Zha et al., 2017) plants the crystal structures of AuNPs were found at 111°, 200°, 220° and 311° peaks. The crystal size of the AuNPs calculated according to the Debye-Scherrer equation (Jacob et al., 2015; Jeyaraj et al., 2019) was determined as 13.40 nm.

$D = K\lambda/(\beta \cos\theta)$

D = size of the particle (nm),

K = Constant (0.89),

λ = Wavelength X-ray (1.5406 Å),

β = Half the value of the highest peak in radians (FWHM=1.267) and

2θ = specified as the angle of refraction (38.053)

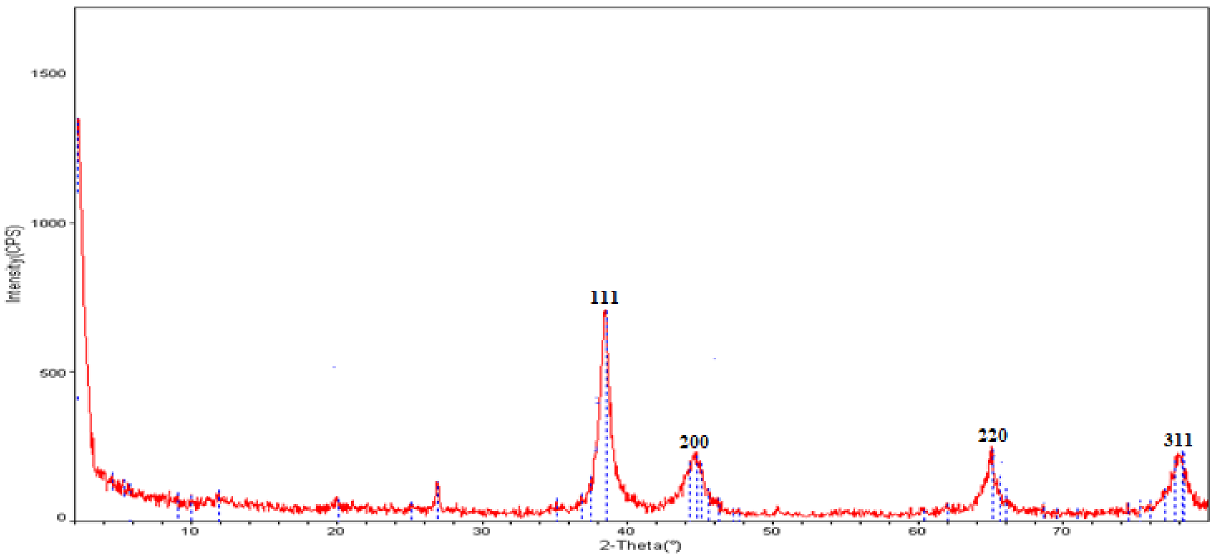

Figure 6. Diagram of XRD and phase analysis of AuNPs.

Thermal gravimetric analysis data

According to the results of the thermal gravimetric analysis - differential thermal analysis (TGA-DTA) (Figure 7), it can be seen from the curve that a 4.68% mass loss at 75.1-224.5 °C was caused by moisture and an 18.35% mass loss at 225-540 °C consisted of organic structures present in the plant extract. The mass loss of 3.26% mass at -1000 °C showed that the nanomaterial was slowly degrading. These results showed that the particles obtained were stable even at high temperatures. The TGA-DTA data of another study, in which nanoparticles were obtained using an eco-friendly method, were interpreted in a similar way to the present study (Gharehyakheh et al., 2020).

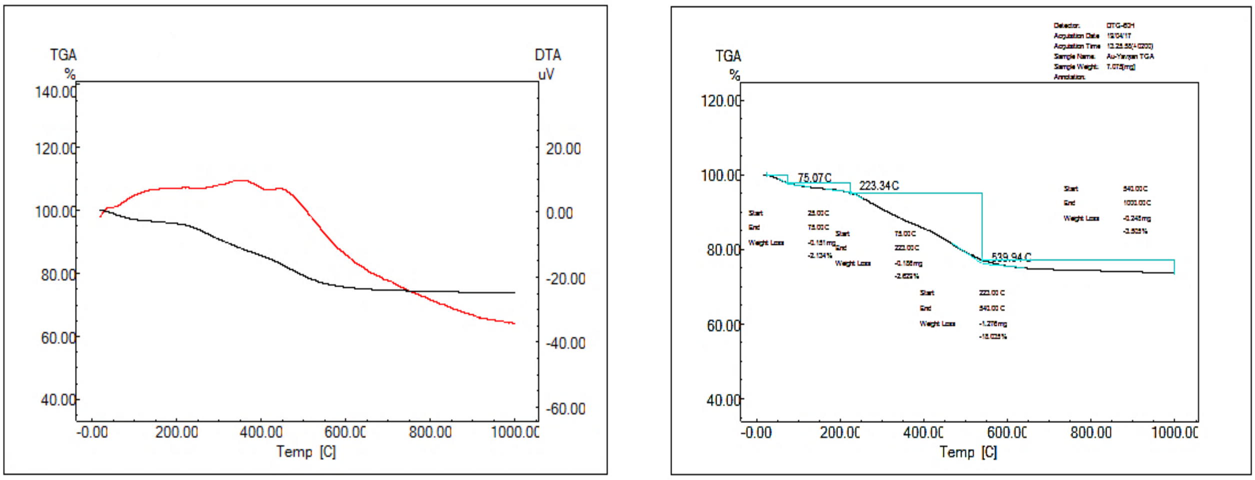

Figure 7. TGA-DTA results of AuNPs synthesized from Wormwood herb

Antimicrobial activities of the AuNPs

The nanoparticles obtained by green synthesis form reactive oxygen species (ROS), which negatively affect cell organelles and the structures in the cell have an affinity for these species (Sun et al., 2019). AuNPs adhere to the cell walls of bacteria and break down the cell membrane. They interact with phosphorus-containing structures, such as proteins and DNA, and negatively affect their function (Gopinath et al., 2016; Pirithiviraj et al., 2016). In this study, the inhibitory effects of the AuNPs obtained on Gram (+) *S. aureus*, ATCC 29213 and *S. pyogenes* ATTC 19615, Gram (-) *E. coli* ATCC25922 and *P. aeruginosa* ATCC 27853 strains and *C. albicans* yeast were examined. The obtained AuNPs were found to have inhibitory effects on Gram (+) *S. aureus*, ATCC 29213 and *S. pyogenes* ATTC 19615, Gram (-) E. coli ATCC25922 and *P. aeruginosa* ATCC 27853 strains and *C. albicans* ATTC 10231 yeast at concentrations of 0.250 and 0.125, 0.125 and 0.033, 0.66 mg mL^{-1}. The AuNPs were found to have a higher concentration effect on gram-positive bacteria due to the thick cell wall structure of these bacteria (Murugan et al., 2015). It has been reported that AuNPs synthesized with *Anacardium occidentale* extract had anti-microbial effects on gram-positive and negative bacteria (Anuradha et al., 2015). In a study regarding green synthesis, it was reported that a 5 mg/L concentration was insufficient for anti-fungal activity (Aravinthan et al., 2017). AuNPs synthesized with *Allium saralicum* have been reported to have a destructive effect on 2 mg/ml of *P. aeruginosa* and *E. coli*, 4 mg/ml of *S. aureus* and 8 mg/ml of *C. albicans* (Huang et al., 2007). When compared to other studies in the literature, it was determined that the AuNPs obtained in the present study had an inhibitory effect on low concentrations of *C. albicans* yeast and other gram (+) and gram (-) bacteria (Table 1).

Table 1. MIC values of synthesized gold nanoparticles (AuNPs) (mg mL^{-1}) on $HAuCI_4$ solution and, *S. aureus*, *S. pyogenes* (vancomycin), *C. albicans* (fluconazole) and *P. aeruginosa*, *E. coli* (colistin) microorganisms.

	Tested Organisms	AuNPs [mg mL^{-1}]	HAuCl4.3H2O [mg mL^{-1}]	Antibiotic [mg mL^{-1}]
Gram (+) bacterial strains	*S. aureus (ATCC 29213)*	0.250	0.5	2
	S. pyogenes (ATTC 19615)	0.125	0.25	1
Gram (-) bacterial strains	*E. coli (ATCC25922)*	0.125	0.25	1
	P. aeruginosa ATCC 27853	0.033	0.1	1
Fungi	*C. albicans (ATTC 10231)*	0.066	0.25	2

CONCLUSION

The interest in ecologically friendly synthesis methods on a global scale is increasing day by day. With the increase in their usage, the synthesis of metallic nanoparticles is becoming more and more significant. AuNPs are used in many medical applications as anti-cancer, anti-microbial and anti-oxidant agents' due to their biocompatibility. In light of this information, in the present study, AuNPs were synthesized with the extract of the *Artemisia absinthium* plant using an eco-friendly, simple and cost-effective method. The nanoparticles showed a suppressive effect at low concentrations on the growth of microorganisms. By improving the synthesis steps, the AuNPs can be used in various medical applications and, due to their stability at high temperatures, they can also be used in different areas that require heat treatment.

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