RESEARCH ARTICLE

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Synthesis, characterization and evaluation of antimicrobial activities of silver nanoparticles obtained from *Rumex acetosella* L. (Sorrel) plant

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Abstract

Rumex acetosella L. (sorrel) is a plant belonging to the Polygonaceous family and is a species that grows naturally across Turkey. In this study, the characterization of silver nanoparticles (AgNPs) obtained from the Rumex acetocella L. (RA) plant using the green synthesis method was performed and their antimicrobial activities were investigated. AgNPs were successfully synthesized in the first stage of the study using plant extract taken from plant samples collected from the natural growing environment. Characterization of synthesized AgNPs was performed using appropriate analytical methods (UV-vis, FT-IR, XRD, SEM-EDX, TEM, Zeta Potential and Zeta Sizer). According to the analysis results, it was determined that AgNPs had a maximum absorbance at 476 nm wavelength, a pentagonal, hexagonal, and spherical appearance, a size of 29.16 nm, and a zeta potential of -9.88 mV. The antimicrobial activities of AgNPs were tested using the microdilution technique, in which Minimum Inhibition Concentration (MIC) values were determined on gram-positive Staphylococcus aureus, Bacillus subtilis and gram-negative Pseudomonas aeruginosa, Escherichia coli bacteria and Candida albicans fungus. It showed a very strong antimicrobial effect on C. albicans, S. aureus and P. aeruginosa. Consequently, AgNPs had stronger antimicrobial activity at low concentrations and when compared to commercial antibiotics.

Keywords: AgNP, Antimicrobial activity, Characterization, Green synthesis. Rumex acetosella L.

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INTRODUCTION

Rumex acetosella L. is a plant belonging to the Polygonaceous family, popularly known as kuzukulağı (sorrel), ebemekşisi, ekşilik, ekşimik, and turşuotu. Studies have reported that Rumex acetosella L. is rich in phenolic compounds that are produced by secondary metabolism in plants. Phenolic compounds, which are synthesized in the cell wall to cope with the ever-changing stress conditions of plants, play a crucial role in reproduction, growth, and metabolism of plants. They function as defense mechanisms against pathological viral and fungal infections, parasites, predators, and environmental factors that may be harmful to the plant (Liu, 2013). Plants accumulate phenolic compounds in their tissues as an adaptive response to adverse environmental conditions, and they have a vital role in regulating various environmental stresses such as excessive light, low temperatures, pathogen infection, herbivores, and nutritional deficiencies. Phenolic compounds are a rich source of antioxidants for the plant due to their binding properties such as the active aromatic core and the hydroxyl groups of the aromatic ring, and the protein-phenol complex, and bring the plant with anti-inflammatory, antitumor, antibacterial, antiviral, and antifungal properties (Naikoo et al., 2019). The Rumex acetosella L. plant is also rich in phenolic compounds, which in turn suggests that it may have antioxidant, anticancer, and antimicrobial activities(Isbilir & Sagiroglu, 2013).

Nanotechnology is a developing science that uses the ability of particles to expand at nanosizes and become more sensitive when compared to its unique counterparts. Nanotechnology has effective application areas ranging from traditional chemical techniques to biological, medical, and environmental technologies. The "green synthesis", namely the synthesis of nanoparticles produced by biological systems refers to the process by which plants or their metabolites combine nanoparticles (Parveen et al., 2016). This new method reduces the toxic effects of NPs produced by conventional (physical and chemical) methods. In addition, green synthesis is preferred as a better alternative to physical and chemical methods in the production of nanoparticles due to its numerous advantages such as simplicity, rapid synthesis, biocompatibility, environmental friendliness and low cost (Atalar et al., 2021; Gour and Jain, 2019). The green synthesis method is commonly used to synthesize metallic nanoparticles such as silver, gold, cadmium, copper, zinc, and platinum. AgNPs stand out among metallic nanoparticles due to their strong, light, catalytic, and antimicrobial properties (Baran et al., 2019, Hatipoğlu, 2022, Hatipoğlu, 2021).

It has been reported that multi-drug resistant bacteria have become one of the most serious public health issues in recent years, causing over 670,000 infections and over 33,000 fatalities per year in the European Union alone. A few species are responsible for the vast majority of these infections and fatalities (Cassini et al., 2019). These species are multi-drug resistant (MDR) Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa bacteria (Kakoullis et al., 2021). Candida albicans, a yeast fungus that causes the majority of infections in humans and is a difficult-to-treat microorganism. In recent years, there has been an upsurge in antibiotic-resistant C. albicans strains, posing a threat to public health (Volkova et al., 2021). To fight against multi-drug resistance, researchers have intensified their studies on next-generation drug design alternatives to traditional antibiotics. Because nanoparticles target several cellular pathways simultaneously, it is extremely difficult for microorganisms to develop resistance to nanoparticles. Therefore, nanoparticles could be an excellent alternative to conventional antibiotics to treat antibiotic-resistant microbial infections(Singh et al., 2020). The aim of the present study is to produce AgNPs by green synthesis method using Rumex acetocella L. plant, to characterize these nanoparticles with appropriate methods and to evaluate their antimicrobial activities against pathogenic microorganisms.

MATERIAL AND METHOD

Plant Specimen

The Rumex acetosella L. (sheep's sorrel) plant was used to synthesize AgNPs. The plant specimen was collected in May 2021 from natural growing zones in the Kızık village of Sandıklı district, Afyonkarahisar province, during its blooming period. The R. acetosella L. plant we collected was cleansed of dust and dirt and allowed to dry in an appropriate room temperature and humidity setting before being used in experimental tests.

Preparation of Plant Extract

After grinding the dried plant parts, 100 g was weighed and placed in a beaker containing 800 mL of distilled water and allowed to boil for 2 hours at 85 °C. The resulting extract after boiling was filtered using Whatman No.1 filter paper, the pulp was discarded, and the resultant plant extract was stored in the refrigerator at +4 °C for use in the next phases.

Preparation of Silver Nitrate (AgNO3) Solution

Silver nitrate (AgNO3) solution (1 mM), which will be used to prepare silver nanoparticles, was prepared using Alpha-aesar brand AgNO3 having an analytical purity of 99.8 %.

Production of AgNPs through Green Synthesis

To synthesize silver nanoparticles, 100 mL of 1mM AgNO3 solution was poured into a beaker, and 20 mL of the previously prepared plant extract was added and left to react at room temperature under constant conditions. The color of the solution darkened after around 150 minutes as a consequence of the transformation of silver ions to AgNPs (Figure 1). The dark solution generated by the reduction of silver ions was centrifuged for 30 minutes at 14000 rpm. Following centrifugation, the liquid portion that had accumulated on the top of the falcon tubes was discarded, and the solid portion at the bottom was washed 10 times with distilled water and centrifuged again until the color of the solution turned clear at the end of the washing procedure. The resultant silver nanoparticles were left to dry at 85°C for 48 hours. After being ground with a glass stirrer, the dried nanoparticles were stored in a dark environment to be used in characterization procedures (Aktepe et al., 2021; Baran and Acay, 2019).

Ultraviolet-visible (UV-vis) Spectroscopy

After mixing the previously prepared AgNO3 solution with the plant extract at a certain rate for the production of AgNPs, measurements were taken at various time intervals in the range of 400 to 800 nm using a UV-1601 220V Shimadzu® model UV-vis Spectrophotometer.

Fourier Transform Infrared (FT-IR) Spectroscopy

In order to determine the functional groups involved in the synthesis of AgNPs and responsible for the decrease in the plant extract, FT-IR analyzes were performed in the range of 4000-400 cm-1 using the Perkin Elmer Spectrum One® model device.

Figure 1. a) Plant extract b) Color change observed during the formation of silver nanoparticles.

X-ray Diffraction (XRD)

The RadB-DMAX II® model computer-controlled X-ray Diffractometer was used to analyze the crystal structure and size of silver nanoparticles at the range of 3°≤ 2θ ≤80°. The average crystal particle sizes of the nanoparticles were calculated using the Debye-Scherrer ($D = K\lambda / (\beta \cos\theta)$) formula (Hatipoğlu, 2021).

Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray (EDX) Spectroscopy

The EVO 40 LEQ model Scanning Electron Microscope (SEM) was used to identify the morphological characterization of the synthesized silver nanoparticles. Energy Dispersive X-ray (EDX) Spectroscopy was utilized to identify the presence of silver in the synthesized nanoparticles and to detect its proportion in the elemental composition.

Transmission Electron Microscopy (TEM)

Jeol Jem 1010® model Transmission Electron Microscope (TEM) was used to determine the morphology and size distribution of nanoparticles.

Zeta Potential and Zeta Sizing

The Malvern® model Zeta Potential device was used to analyze the surface charge distribution and estimated size of AgNPs.

Determination of Antimicrobial Activities of Synthesized AgNPs

The antimicrobial activities of AgNPs produced from the Rumex acetosella L. plant using the green synthesis method were assessed using the microdilution method, which indicated the lowest concentration (MIC) required for prevention of microorganism growth. The MIC values of AgNPs produced from the plant extract, 1 mM silver nitrate solution (AgNO3), and commercial antibiotics were analyzed to make these assessments.

Microorganisms tested for antimicrobial activity

To determine the antimicrobial activities of AgNPs, cultures of gram-positive bacteria S. aureus (ATCC 29213) and B. subtilis (ATCC 11774), gram-negative bacteria P. aeruginosa (ATCC 27853) and E. coli (ATCC 25922) and the fungus C. albicans (ATCC 10231) were selected, all of which were incubated overnight. The plates were then incubated. 24 hours at 36°C in an incubator. antibiotics and AgNO3 MIC values were determined(Keskin and Güvensen 2022).

RESULTS AND DISCUSSION

Characterization of Silver Nanoparticles

Analysis of UV-vis Spectroscopy

Identical optical properties of metallic nanoparticles interact with light, resulting in the appearance of the surface plasmon resonance (SPR) band. AgNP production is indicated by the reduction of Ag+ ions to Ag0 by oxidized plant components and the color of the solution turning from yellow to brown as a consequence of vibrations (SPR) on the plasma surface. Nanoparticle stability is determined using UV-vis spectroscopy. Due to the plasmon resonance of AgNPs, the absorption spectra generating on the surface of AgNPs have an absorption capacity of 425 to 475 nm (Banerjee et al., 2014).

In the present study, the addition of 10 mM AgNO3 solution at a 3:7 ratio to the plant extract, which was originally yellow, caused a color shift from yellow to brown in a short period of time. Following that, UV-vis measurements at 30th, 60th, and 90th minutes determined that AgNPs produced by vibrations on the plasma surface exhibited maximum absorbance at 476 nm (Figure 2). This finding corresponds to the data in previous studies. A similar study by Baran, M. F. et al., (2019) reported that AgNPs produced from olive leaf extract had a maximum absorbance value of 468 nm (Baran et al., 2019).

Analysis of Fourier Transform Infrared Spectroscopy

FT-IR analyses were conducted (Figure 3a, 3b) to identify possible functional groups in plant phytochemicals that play a role as reducing and stabilizing agents in AgNPs synthesis, and the FT-IR spectrum of R. acetocella L. extract and the synthesized AgNPs were compared. It represents the peak -OH (hydroxyl) functional group at 3338 cm-1, the peak -C≡N functional group at 2114 cm-1, and the peak -C=O (carbonyl) functional group at 1635 cm-1 seen in the spectra. It is possible to assert that the reaction took place in these groups due to the frequency shifts in these peaks. Acay et al., reported similar functional groups in the characterization of AgNPs produced by using Vitis vinifera leaf extract (Acay & Baran, M. F., 2019), which is compatible with the present study. Baran A., et al. (2022) also reported the existence of -C=O and -OH groups in their study on the leaves of the Cicer arietinum plant.

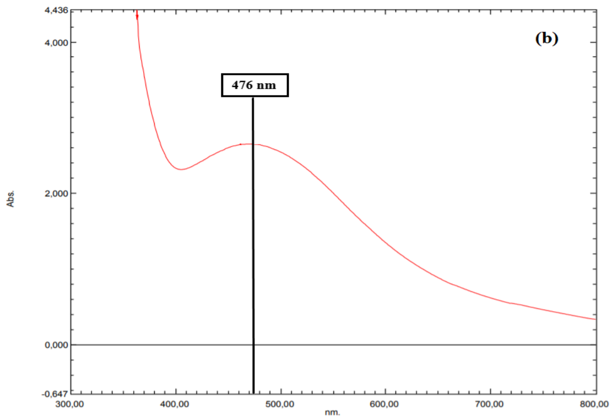

Figure 2. Maximum absorbance value of AgNPs synthesized by Rumex acetosella L. plant in UV-vis spectroscopy

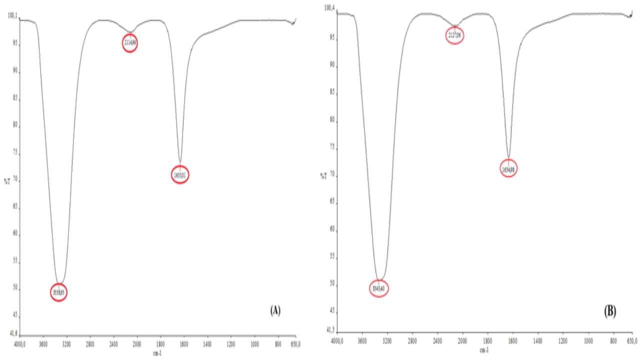

Figure 3. Functional groups involved in reduction by FT-IR analysis: a) FT-IR spectrum of Rumex acetosella L. plant extract, b) FT-IR spectrum of synthesized AgNPs

Analysis of X-ray Diffraction

XRD analysis was done to examine the crystal structure and size of AgNPs produced from the Rumex acetosella L. plant by the green synthesis method. Figure 4 shows the X-ray Diffraction pattern of AgNPs. The peak reflections on Bragg diffraction plans (111), (200), (220) and (311) were calculated with values corresponding to 2θ (38.18°, 46.31°, 64.47° and 77.46°) in the XRD spectrum of the synthesized nanoparticles. The average crystal size of AgNPs was determined to be around 29.16 nm by using the Debye-Scherrer formula (D = Kλ/ (β cosθ). Similarly, Adil et al., (2019) reported a crystal size of 22.48 nm for AgNPs produced from the Hypericum triquetrifolium Turra plant.

Analysis of Scanning Electron Microscopy and Energy Dispersive X-ray Spectroscopy

SEM is a powerful electron microscope and a popular surface imaging technique. SEM produces a high-resolution, magnified three-dimensional image and provides information on the purity of the sample (Atalar et al., 2021). It magnifies images using electrons rather than light, as conventional microscopes do (Sharma et al., 2019). This microscobe was used to analyze the surface morphology of AgNPs produced from the R. acetosella L. plant. When the SEM images were examined, the silver nanoparticles exhibited a spherical structure, as seen in Figure 5.

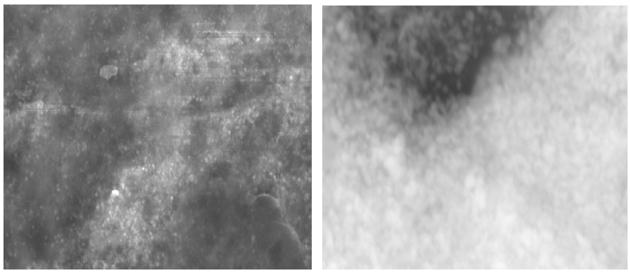

Figure 5. SEM images of AgNPs synthesized by Rumex acetosella L. plant

EDX Spectroscopy was used to validate the presence of silver (Ag) in the elemental composition and to identify other elemental compositions. According to the EDX analysis, Ag appeared to emit a strong signal in the silver area, indicating that there was a significant quantity of Ag in the element composition and therefore verifying the production of AgNPs (Ma et al., 2016). Furthermore, the presence of compounds in Rumex acetosella L. extract may cause weak signals from Chlorine (Cl), Carbon (C), and Oxygen (O) determined by EDX data (Figure 6). Consistent with the findings of the present study, Azhdari et al. (2020) reported that silver nanoparticles synthesized from the Stachys lavandulifolia plant exhibited a spherical morphology as verified by SEM and they had a high proportion of silver in elemental composition as determined by EDX.

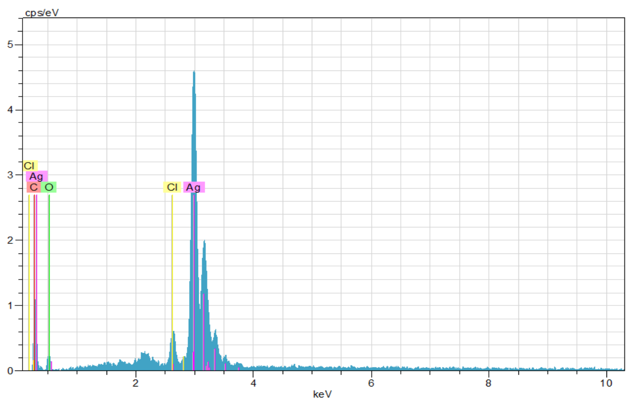

Figure 6. Elemental composition of AgNPs synthesized by Rumex acetosella L. plant by EDX

TEM Analysis

The morphological appearance and size of AgNPs synthesized from R. acetosella L. plant were analyzed using TEM. TEM analysis revealed that the nanoparticles were of numerous morphologies, including pentagonal, hexagonal, and spherical (Figure 7). The findings of this study are consistent with the research of Banerjee et al. (2014), who reported triangular, pentagonal, hexagonal and spherical silver nanoparticles produced from Musa balbisiana (banana), Azadirachta indica (neem) and Ocimum tenuiflorum (black tulip).

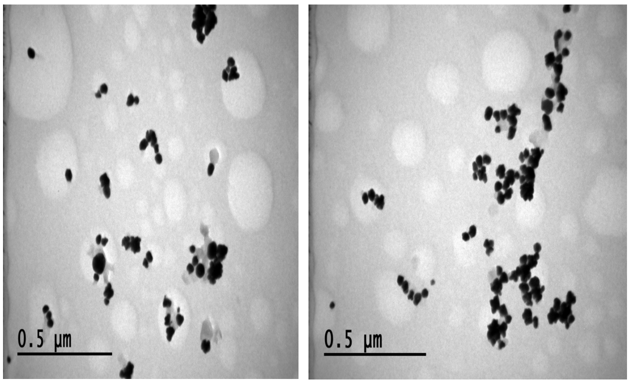

Figure 7. TEM images of AgNPs synthesized by Rumex acetosella L. plant

Analysis of Zeta Potential and Zeta Sizing

The zeta potential, also known as the electrokinetic potential, is a measure of charge stability that indicates the stability of colloidal nanoparticles. It controls every interaction between particles in a suspension. The zeta potential has values ranging between +100 mV and -100 mV. When the zeta potential is more than +30 mV or less than -30 mV, it indicates that a suspension is significantly stable. The zeta potential depends on the velocity of the particle moving under the influence of the electric field and the viscosity of the dispersion medium (Naser et al., 2020). Zeta potential and Zeta sizing analyses were used to determine the surface charge distribution and approximate size of AgNPs synthesized using the green synthesis method. According to the results of the zeta analysis, the electrical charge of the nanoparticles was determined as 9.88 mV (Figure 8a). The negative electrical charge of AgNPs indicates that there is no aggregation in the suspension and the nanoparticles have a stable structure. As shown in Figure 8b, the size of the resultant silver nanoparticles was determined to be in the range of 25.59-124 nm. Likewise, Varadavenkatesan et al. (2017) reported that the electrical charge of AgNPs synthesized from Vigna mungo was negative and the average size of these silver nanoparticles was in the range of 28.21-91.28 nm..

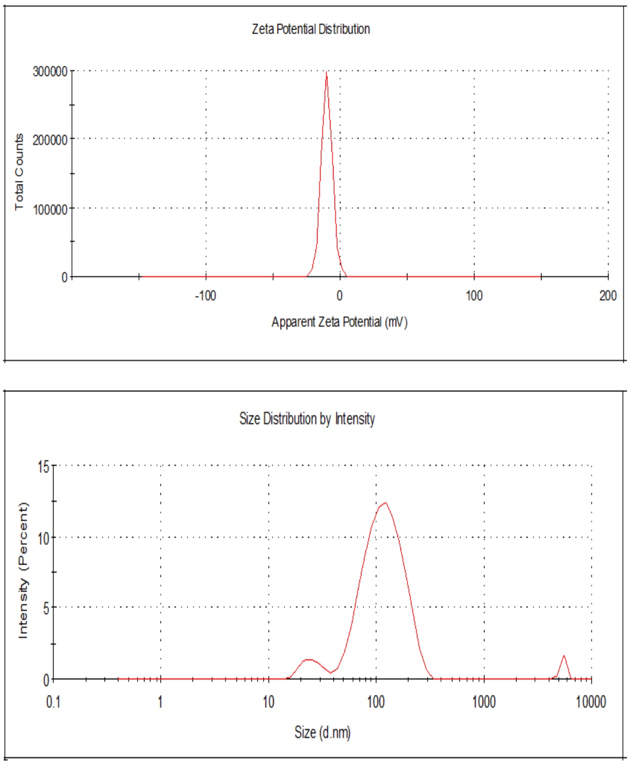

Figure 8. a: Zeta potential plot of AgNPs synthesized by Rumex acetocella L. plant b: Zeta size plot of AgNPs synthesized by Rumex acetocella L. plant

Assessment of Antimicrobial Activities of Silver Nanoparticles

In the present study, the possible antimicrobial effects of AgNPs produced by the green synthesis method from the Rumex acetosella L. plant on gram-positive (S. aureus ATCC 29213, B. subtilis ATCC 11774) and gram-negative (P. aeruginosa ATCC 27853, E. coli ATCC 25922) bacteria and C. albicans ATCC 10231 fungus, which have pathogenic effects on humans, were evaluated. The MIC values were measured using a 1 mM silver nitrate solution (AgNO3) and commercial antibiotics for positive control in order to make comparison with AgNPs. The findings suggested that AgNPs had antimicrobial activity even at low concentrations, and it was more effective than AgNO3 and antibiotics.

When the data in Table 2 and Figure 9 were examined in the present study, AgNPs demonstrated an antibacterial effect on Gr+ bacteria at concentrations of 0.125 µg mL-1 for S. aureus (ATCC 29213) and 0.250 µg mL-1 for B. subtilis (ATCC 11774). According to the MIC values, AgNPs were found to be roughly twenty times more effective on S. aureus and five times more effective on B. subtilis than silver nitrate and vancomycin antibiotic employed as a positive control. These data indicated that S. aureus bacteria were more sensitive to AgNPs than B. subtilis bacteria.

Table 2. MIC values (µg mL1) showing the antimicrobial activities of AgNPs, silver nitrate and antibiotics

	Microrganisms	AgNPs µg mL-1	AgNO3 µg mL-1	Antibiotic µg mL-1
Gram (+) Bacteria	*S. aureus*	0.125	2.65	2
	ATCC 29213			
	B. subtilis	0.250	1.32	1
	ATCC 11774			
Gram (-)Bacteria	*E. coli*	1.00	0.66	2
	ATCC 25922			
	P. aeruginosa	0.50	0.66	2
	ATCC 27853			
Yeast	*C. albicans*	0.012	0.66	2
	ATCC 10231			

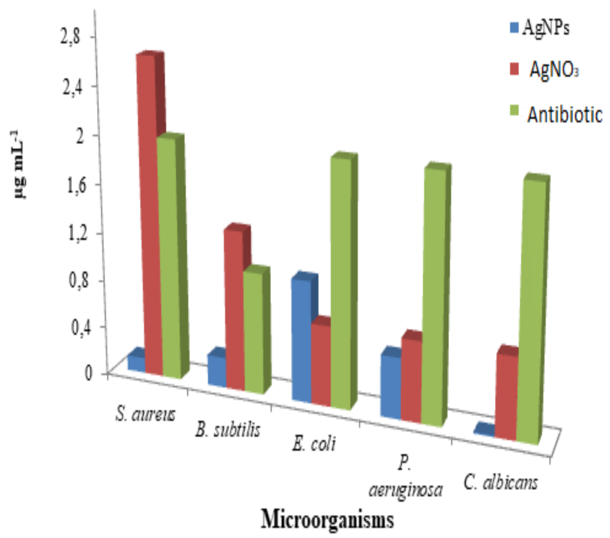

Figure 9. MIC values (µg mL-1) of AgNPs, antibiotics and silver nitrate against the microorganisms

MIC values of Gr- bacteria indicated that AgNPs exhibited an antibacterial effect at concentrations of 1.00 µg mL-1 for E. coli (ATCC 25922) and 0.50 µg mL-1 for P. aeruginosa (ATCC 27853). Based on these findings, it was determined that AgNPs were more efficient against E. coli and P. aeruginosa than the colistin antibiotic employed as a positive control and exhibited almost the same effect as silver nitrate solution. P. aeruginosa bacteria are also observed to be more sensitive to AgNPs than E. coli bacteria.

These results indicated that AgNPs exhibited more antimicrobial activities against gram-positive bacteria than gram-negative bacteria, and that gram-positive bacteria were more sensitive to AgNPs than gram-negative bacteria. Gram-negative bacteria become more negatively charged than gram-positive bacteria due to a lipopolysaccharide layer on the outer surface of their cell wall. Therefore, we think that the electrostatic repulsive force between negatively charged AgNPs and gram-negative bacteria inhibited partially AgNPs from penetrating the bacterial cell.

AgNPs were observed to have a considerable antifungal effect on C. albicans (ATCC 10231), one of the pathogenic fungal species, at a concentration of 0.012 g mL-1. Based on this MIC value, AgNPs were found to be fifty-five times more effective than silver nitrate and one hundred sixty-six times more effective than the fluconazole antibiotic employed as a positive control on C. albicans. At this point, it was concluded that the phytochemicals in the content of the examined plant were much more effective in terms of antifungal activity.

A similar study reported that AgNPs produced from Cynara scolymus L. (artichoke) plant by green synthesis method were effective on S. aureus, B. subtilis, P. aeruginosa, E. coli and C. albicans at concentrations of 0.12, 0.25, 0.07, 0.13, 0.03 µg mL-1, respectively. In the same study, AgNPs were reported to be effective at lower concentrations than silver nitrate and antibiotics (Baran et al., 2021). In another similar study, it was reported that AgNPs synthesized from Punica granatum (pomegranate) leaves showed antibacterial activity at 0.050-0.125 mg mL-1 concentrations on E. coli, P. aeruginosa, S. aureus, B. subtilis and Proteus vulgaris bacteria (Singhal et al., 2021). AgNPs synthesized from the Zea mays L. (corn) plant were reported to exhibit antimicrobial effects on E. coli, S. aureus bacteria, and C. albicans fungus at concentrations of 0.084, 0.337, and 0.021 µg mL-1, respectively (Eren & Baran, 2019). MIC values of AgNPs synthesized from Hypericum triquetrifolium Turra (hypcricum) plant against E. coli and S. aureus bacteria and C. albicans fungus were reported as 0.041, 0.662 and 0.020 µg mL-1, respectively (Adil et al., 2019). The effective concentrations of AgNPs synthesized using Euphorbia hirta (asthma plant) on S. aureus and E. coli bacteria were reported to be 0.82 and 0.67 µg mL-1, respectively (Kumar et al., 2016). All of these studies suggest that AgNPs produced from various plants using the green synthesis method may have antimicrobial effects against the same pathogenic microorganisms at different concentrations.

CONCLUSION

Antibiotic resistance to commonly used pharmaceuticals poses a threat to human health. Bioactive compounds and nanoparticles synthesized by plants are a goldmine in the fight against multidrug-resistant microorganisms and may be used as alternatives to traditional medications. The fact that the strong inhibitory effect of AgNPs synthesized by R. acetosella on these pathogenic microorganisms was higher than similar studies suggests that it would contribute to the development of antibiotics against the agents of infections caused by resistant microorganisms. Further studies are required 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.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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