Applied Biochemistry and Biotechnology (2024) 196:1089–1103
https://doi.org/10.1007/s12010-023-04592-w

ORIGINAL ARTICLE

Investigation of Antibiofilm and Antibacterial Properties of Green Synthesized Silver Nanoparticles from Aqueous Extract of *Rumex* sp.

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Accepted: 26 May 2023 / Published online: 17 June 2023

Abstract

The decrease in the effectiveness of conventional drugs as a result of the growth of resistance to antibiotics has increased the need for innovative tools to control the infections. At this point, metallic nanoparticles, in particular silver nanoparticles, have appeared as a promising method. In the current study, the extract of *Rumex* sp. (Labada, dock) leaves was used as a reducing agent for the formation of silver nanoparticles. Unlike similar studies, in this study the synthesis conditions were optimized by changing the extract ratio and silver nitrate concentration. Morphological investigations of synthesized silver nanoparticles showed that spherical homogeneous particles at size under 100 nm had been produced. SEM/EDS and FTIR analyses showed that plant components are involved in the synthesis of nanoparticles. It was also determined that higher extract ratio reduced nanoparticle size. The antimicrobial effects of the synthesized nanoparticles against *Gram-positive* and *Gram-negative* bacteria were tested, and it was determined that all nanoparticles exhibited activity against both groups. *Rumex* sp. silver nanoparticles (NPs) were revealed to exhibit antibiofilm activity against three different isolates with moderate and strong biofilm-forming ability. The NPs reduced the biofilm-forming capacity of *Acinetobacter baumannii* and *Klebsiella pneumonaie* by 2.66-fold and 3.25-fold, whereas they decreased the *Escherichia coli* biofilm-forming capacity by 1.25-fold. The investigation of microbial biofilm could play an important role in developing new strategies for treatment options. Our results suggest that *Rumex* sp. silver NPs may have a high potential for use in the treatment of pathogenic strains.

Keywords Antibiotic · Biofilm · Green synthesis; Nanotechnology · Silver nanoparticle

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Introduction

Antimicrobial activity is associated to compounds that are able to kill or slow down microorganisms, without any toxic effects. Although these agents are crucial to combat infectious diseases, due to their extensive and misuse, bacterial resistance to the drugs has arisen over decades and become a common problem [12]. In spite of the obtainability of numerous broad-spectrum antimicrobial drugs, common infections stand as the major emergency worldwide due to the resistance to most of the common antibacterial agents. Even though there is momentous improvement in the treatment of many infectious diseases by microorganisms and viruses, the quantity and spreading of pathogenic resistant microorganisms have also amplified dramatically. Two million individuals are affected from infections with bacterial resistance annually, and it is foreseen that the worldwide deaths will pass 10 million annually until 2050. The market size of antimicrobial compounds is also expected to reach 20.50 billion USD by 2026. While the necessity for antibiotics is constantly growing over time, scientists have been working around the world to find a sustainable solution. Numerous novel alternatives, such as herbal the plant extracts, natural proteins, and bacteriophages, have been presented to strengthen the combat against pathogenic microorganisms. Among the proposed alternatives, metallic nanoparticles, especially silver, have stood out due to their improved bioavailability, high antimicrobial effectiveness, less toxicity, and specific targeting, compared to other methods [1, 18, 26, 31]. During the past years, nanotechnology has been expected to have a substantial influence on society by nano-based breakthrough progresses in biotechnology, medicine, nanoelectronics, as well as in materials and manufacturing areas [18]. Nanoparticles have been regarded as particles whose size is below 100 nm and have unique features such as optical, electrical, and thermal properties as a result of the enhanced surface area/volume ratio [3, 5, 27, 29, 31].

Handling nanotechnology with green chemistry principles is a flexible approach and has widened its popularity in the recent years [2]. In this approach, nanotechnology is combined with green chemistry to form nanomaterials in a cost-effective and safe fashion. In the green synthesis of silver nanoparticles, the nanocrystals are typically generated from Ag^+ ions which are provided by salts like silver nitrate ($AgNO_3$. On the other hand, biological materials, microbes, or extracts of diverse plants can be used as reducing agents. Reducing agents in the solution allow the ions to reduce to atoms. Afterward, the atoms are gathered into clusters and form nanoparticles. Therefore, the presence of atoms depends on the concentration of silver salt and reducing agent, which also needs to be optimized to regulate the morphology and size of the nanoparticles [1, 25].

The applications of silver nanoparticles can be distributed in several fields, mostly pharmaceutical and medical areas due to their reduced toxicity and enhanced stability. Silver nanoparticles have not only been used as antimicrobial agents, but also have been revealed as a superior vehicle to avert viruses binding to host cells [30, 31] and stop mosquito vectors as a green mosquitocidal [22]. Silver nanoparticles (AgNPs) are shown to kill bacteria by ability to neutralizing the membrane's surface and changing the penetrability. Reactive oxygen species (ROS) generation which leads to deformation of the cell membrane and intracellular effects, such as interactions with proteins and nucleic acids, are the other main pathways behind the antibacterial actions of nanomaterials [2, 18, 31]. The antimicrobial mechanism of silver containing nanoparticles is attributed to several mechanisms mostly based on Ag^+ ions., These Ag^+ ions are generated when dissolved in an aqueous solution. Ag^+ is able to bond to negatively charged fragments of the cell membrane that leads a formation of holes in the membrane. The most advantageous property of the silver nanoparticles is that, due to various mechanisms of antimicrobial action, resistance to them has been rare and slow, compared to resistance to antibiotics [24, 31]. Nanoparticles (NPs) are also promising as a means of treating bacterial biofilms. In addition to the inadequacy of bacterial antibiotic-resistance mechanisms against NPs, the electrostatic interaction between NPs and biofilms is effective in overcoming biofilm-based resistance mechanisms. Some NPs have been reported to be effective against many biofilm-forming infectious agents [24]. Although a number of NPs are applied as antimicrobial agents, among the most potent in the prevention of severe infections are silver NPs (AgNPs).

Many types of extracts derived from plants have been recognized as non-hazardous reducing agents for nanoparticle formation. Because the plant parts contain a variety of compounds for silver reduction, the technique is inexpensive, environmental-friendly, and high efficient. Therefore, the synthesis of silver nanoparticles using such agents has attracted various researchers [17].

Medicinal plants have been utilized as sources due to their various natural products contents, which show bioactivity to defend the body against both cellular defects and pathogens [7]. The genus Rumex (*Polygonacea*) is broadly distributed in flora of Turkey, and most widespread species are *Rumex patientia* L., *Rumex crispus* L., *Rumex acetosa* L., *Rumex caucasicus*, and *Rumex alpinus* L., *R. alpinus*, and *R. caucasicus* are both known as “evelik” or “labada” in local and distributed eastern Anatolia, mostly at higher altitudes. These herbs have been used widely as traditional medicine in Turkey against various disorders due to their anti-inflammatory, diuretic, laxative, antipyretic, and wound healing properties. Also, fresh leaves of *Rumex* spp. are consumed as a vegetable or for seasoning meat or cheese in the eastern part of Turkey [23]. Recently, there are several studies reporting the green synthesis of silver nanoparticles using various species of *Rumex* [4, 11], however, there is still need for investigating various bioactivities of green synthesized nanoparticle using *Rumex* extracts.

The main goal of this study is to optimize the conditions for production of silver nanoparticles by green synthesis using the aqueous extract of *Rumex* sp. leaves and to provide a simple and sustainable method. The main contribution of the study, contrary to other reports, is that the optimum conditions for silver nanoparticle formation were determined by applying various parameters such as extract ratio and silver nitrate concentration and also the effects of these factors on morphology, size, and structure of nanoparticles, and were revealed. The antibiofilm potential of produced nanoparticles on clinical isolates has been also determined. The antibiofilm activity is another contribution of the study due to the lack of this test in similar reports.

Materials and Methods

Preparation of the Herbal Plant Extracts

Leaves of *Rumex* sp. were collected from the Kars Province, the northeastern part of Turkey. For the preparation of the plant of extracts previously reported, protocol was modified and used [23]. The leaves were dried at room temperature and ground in the mortar to form a fine powder. Five grams of leaf powder was extracted with 200 mL of water in a Soxhlet under reflux for an hour. The plant pulp was separated through filtration using filter paper and, the extracts were stored at 4 °C for further studies. The extracts were also centrifuged for 5 min at 4500 rpm to eliminate possible residues. The supernatant was used in the construction of nanoparticles.

Synthesis and Optimization of Silver Nanoparticles

For the preparation of silver nanoparticles, leaf extracts have been used in reducing silver nitrate ($AgNO_3$) at varying concentrations. To determine the optimum conditions for nanoparticle synthesis, the extract to $AgNO_3$ ratio (v/v) was also examined. In the synthesis procedure, a certain volume of $AgNO_3$ (Table 1) has been kept constant, and the extract was added dropwise at a constant rate under stirring and left for 4 h at room temperature.

At the end of the duration, the mixture has been centrifuged at 12000 rpm for 10 min to remove non-particle components, and the silver nanoparticles were separated from the reaction mixture. This step was repeated 2 times with distilled water and with ethanol to clean unwanted residues from nanoparticles. The produced nanoparticles were left to dry at room temperature for further characterization. The experimental set points and conditions are given in Table 1.

Characterization of Synthesized Silver Nanoparticles

UV-Vis Spectroscopy Analysis The visual characterization of silver nanoparticles was first observed by changes in the color of the reaction mixture. The UV-visible absorbance of samples was measured using a UV-Vis spectrometer run at a resolution of 1 nm with a range between 300 and 800 nm.

FTIR Analysis Fourier transform infrared spectroscopy (FTIR) analysis was performed to reveal the involvement of plant extract-derived compounds in the synthesis silver nanoparticles as reducing agents. FTIR spectra of silver nanoparticle and *Rumex* sp. leaf extracts were recorded in the in a range of 450–4000 cm^{-1}.

Scanning Electron Microscopy Inspection The morphological information and surface properties of the synthesized silver nanoparticles were obtained through scanning electron microscopy equipped with energy dispersive spectroscopy (SEM/EDS, Zeiss Sigma 300). The particle size distribution was determined by analyzing the images of SEM/EDS.

Antimicrobial Activity of Synthesized Silver Nanoparticles

The agar well diffusion technique was used to investigate the antimicrobial activity of nanoparticles and nanoparticles were tested against 2 *Gram-negative* (*Pseudomonas aeruginosa* and *Escherichia*

Table 1 Experimental set points for nanoparticle synthesis

Sample	Extract ratio	$AgNO_3$ C (mM)	Extract V (mL)	$AgNO_3$ V (mL)	Drop rate (mL/min)	Time (h)
1	5%	2.5	5	95	0.5	4
2		5	5	95	0.5	4
3		7.5	5	95	0.5	4
4		10	5	95	0.5	4
5	10%	10	10	90	0.5	4
6		5	10	90	0.5	4
7		7.5	10	90	0.5	4
8		2.5	10	90	0.5	4

coli) and 2 *Gram-positive* (*Bacillus subtilis* and *Staphylococcus aureus*) bacteria. Six millimeter-diameter wells were opened on the agar layer in the petri dishes, and 80 µL of samples to be tested was added to the wells (0.5 mg/mL), subsequently left for incubation at 37 °C overnight. The same amounts of ampicillin (50 µg/mL), leaf extract, and $AgNO_3$ (10 mM) were used as control. The antimicrobial activity of samples was assessed by measuring the inhibition zone diameters.

Determination of *Rumex* sp. silver nanoparticle MIC via broth microdilution

The minimum inhibitory concentration (MIC) of *Rumex* sp. silver NPs was determined by liquid microdilution. The NPs at the concentration range of 500–67.5 µg/mL were prepared, and experiments were performed in triplicate using a 96-well plate. *Escherichia coli* DH5a was used as the control. The determined MIC values were used as reference in the investigation of the effect on biofilm formation of the strains (*A. baumannii*, *E.coli*, and *K. pneumoniae*) [8].

Effect of *Rumex* sp. silver nanoparticles on biofilm formation

The study investigated the effect of the NPs on biofilm formation in medium and strong biofilm-forming strains. After determining the MIC values of the *Rumex* sp. silver NPs on the strains as 125 µL/mg, 67.5 µL/mg concentration was used for the biofilm formation experiments. Experiments were performed in triplicate on 96-well plates. And then quantitative determination of biofilm formation was performed using the previously mentioned method [8, 14].

Results

Synthesis and Optimization of Synthesized Silver Nanoparticles

The absorption wavelengths and the color are easy ways to reveal the formation of silver nanoparticles. As the reaction progresses and silver nanoparticles are formed, the color of the reaction medium changes from yellow to dark brown. The color of the reaction mixture changed in very few minutes upon the initiation of the reaction. Figure 1(A) shows the changes in the color for each hour following the start of the reaction. This observation was also supported by maximum wavelength measurements. As presented in Fig. 1(B), the noticeable peak was recorded between 400 and 500 nm, which indicated the existence of silver nanoparticles. The intensity of the peak as well as the color was observed to get higher in the course of the reaction period as expected due to the increase in silver nanoparticle amount in the reaction media (Fig. 2). These findings are consistent with literature studies [10, 27].

FTIR Analysis

FTIR analysis of both *Rumex* sp. extract and synthesized AgNPs was performed to find the natural constituents which might be involved in the reduction of silver nanoparticles.

FT-IR spectra demonstrated similar intense peaks at 3202, 2916, and 1586 cm^{-1}, as shown in Fig. 3, which indicated the stretching vibration for N–H, aliphatic C–H, and C=O respectively. Other strong peaks at 1405 and 1007 cm^{-1} also show the presence of

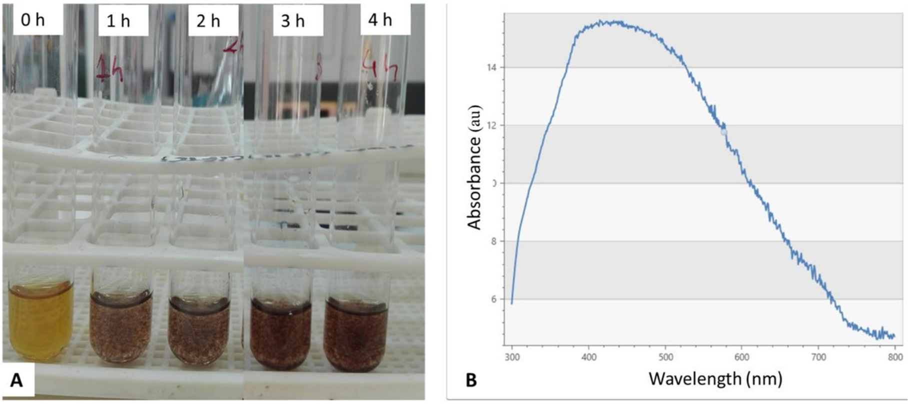

Fig. 1 **A** Color change during the reaction; **B** UV spectrum of synthesized silver nanoparticles after 4 h

the stretching vibration bands of C–N and symmetric C–O groups (O = C–O, C–O–C, and epoxide), respectively [4]. Regarding the difference between the spectra, it can be concluded that the emergence and intensity of some peaks (3202, 2916, and 1586 cm^{-1}) confirms the stabilization of silver nanoparticle by functional groups in plant extracts. These primary functional groups are present in most natural compounds such as phenols and flavonoids [4, 7]. These findings proved that the natural constituent of *Rumex* sp. leaves act as capping agent for silver nanoparticle synthesis.

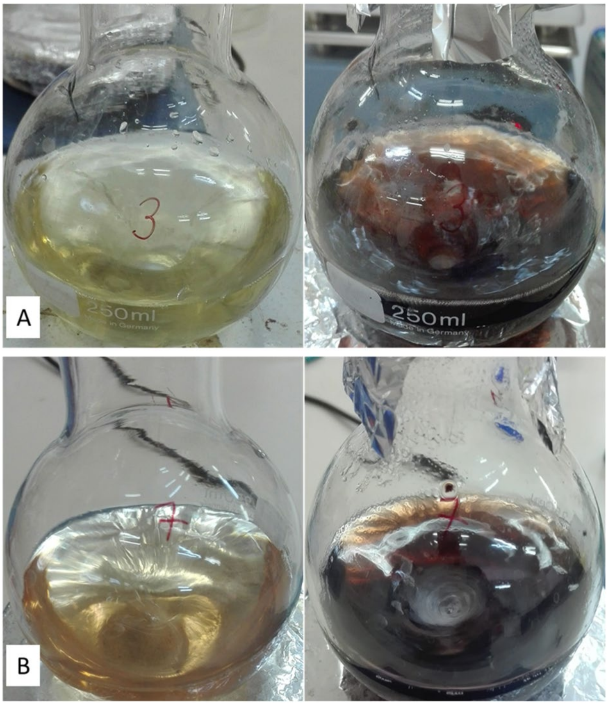

Fig. 2 Color change which indicates the formation of nanoparticles during the reaction **A** 5% extract ratio; **B** 10% extract ratio. The $AgNO_3$ concentration is 7.5 mM for both samples (Samples 3 and 7)

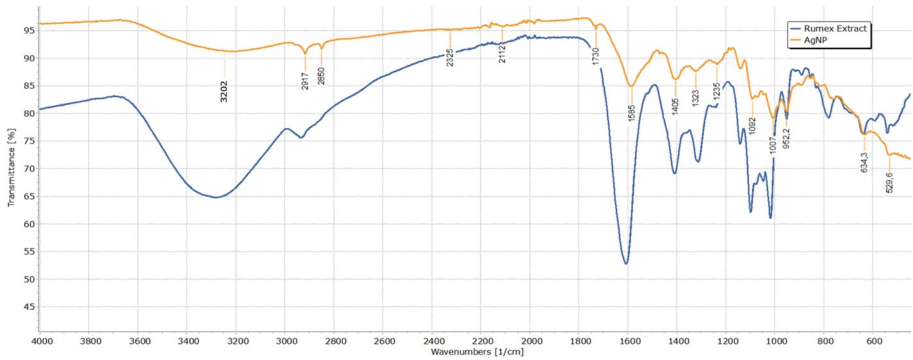

Fig. 3 FTIR spectrums of AgNPs and *Rumex* sp. extracts

SEM/EDS Analysis of Silver Nanoparticles

The morphological inspection and elemental analysis of produced silver nanoparticles were explored using SEM coupled with an EDS (energy dispersive X-ray spectrometer). The SEM investigation showed that nanoparticles were quite homogenous (Figs. 4 and 5). The size determination also confirmed that all nanoparticles were below 100 nm.

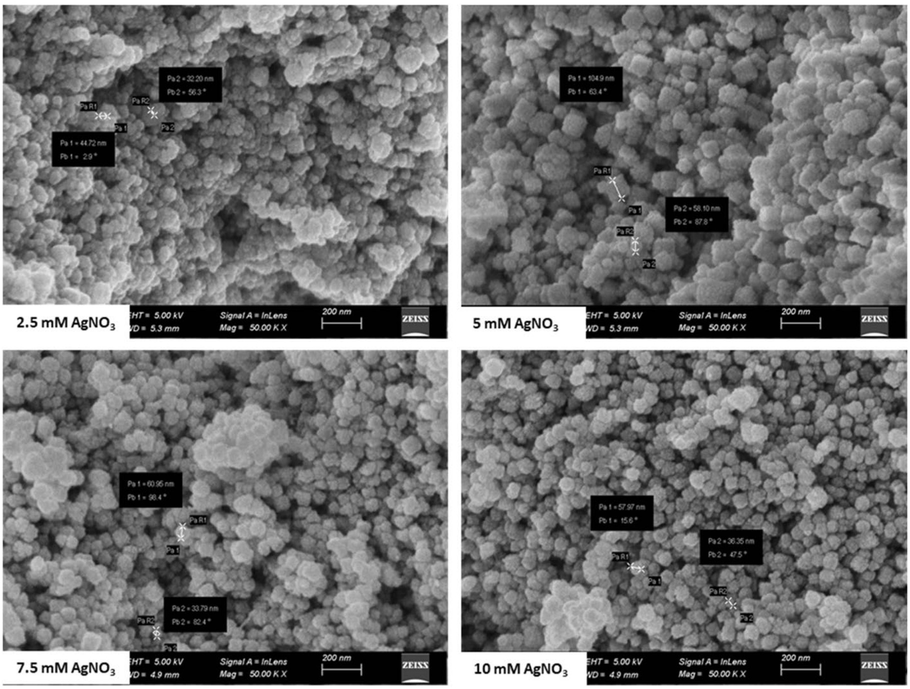

Fig. 4 SEM images of silver nanoparticles produced with 5% extract ratio under various $AgNO_3$ concentrations

Considering the SEM analysis, the size of produced silver nanoparticles ranged from 10 to 100 nm, and the smallest size was obtained in experiments performed with 10% extract ratio. Regarding morphological structures of the synthesized nanoparticles, it was determined that the majority of them were spherical, yet hexagonal and cubic-shaped nanoparticles were also produced. The SEM images also showed that increasing the plant extract ratio caused the produced nanoparticles smaller in size [15, 32].

EDS images (Fig. 6) indicated strong signals for silver metal in all samples which approved the presence of silver as a main component of nanoparticles (in the range of 50–83%). It has been observed that the oxygen content is increasing with the increase in extract ratio which indicates that some of the silver nanoparticles are in the oxide form.

Antimicrobial Activity of Silver Nanoparticles

The antimicrobial activity of green synthesized silver nanoparticles was investigated against *E. coli*, *P. aeruginosa*, *S. aureus*, and *B. subtilis* by agar well diffusion assay. The results showed that all produced nanoparticles had almost similar activity against both *Gram-positive* and *Gram-negative bacteria* (Fig. 7). However, there was no activity exhibited against *B. subtilis*. The diameter of inhibition zones is presented in Table 2. Although all nanoparticles showed antibacterial activity, the samples synthesized with higher extract ration and higher $AgNO_3$ concentration (Sample 8) showed slightly higher inhibition, which is probably because these nanoparticles had relatively smaller size. A crucial observation of the current study can be expressed as follows: the crude plant extract exhibited any inhibition

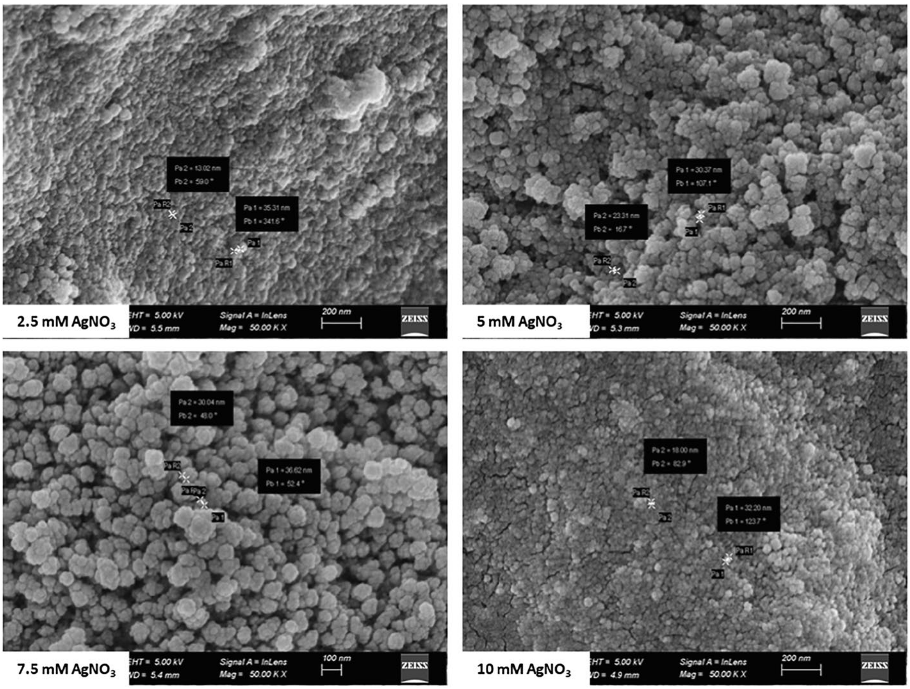

Fig. 5 SEM images of silver nanoparticles produced with 10% extract ratio under various $AgNO_3$ concentrations

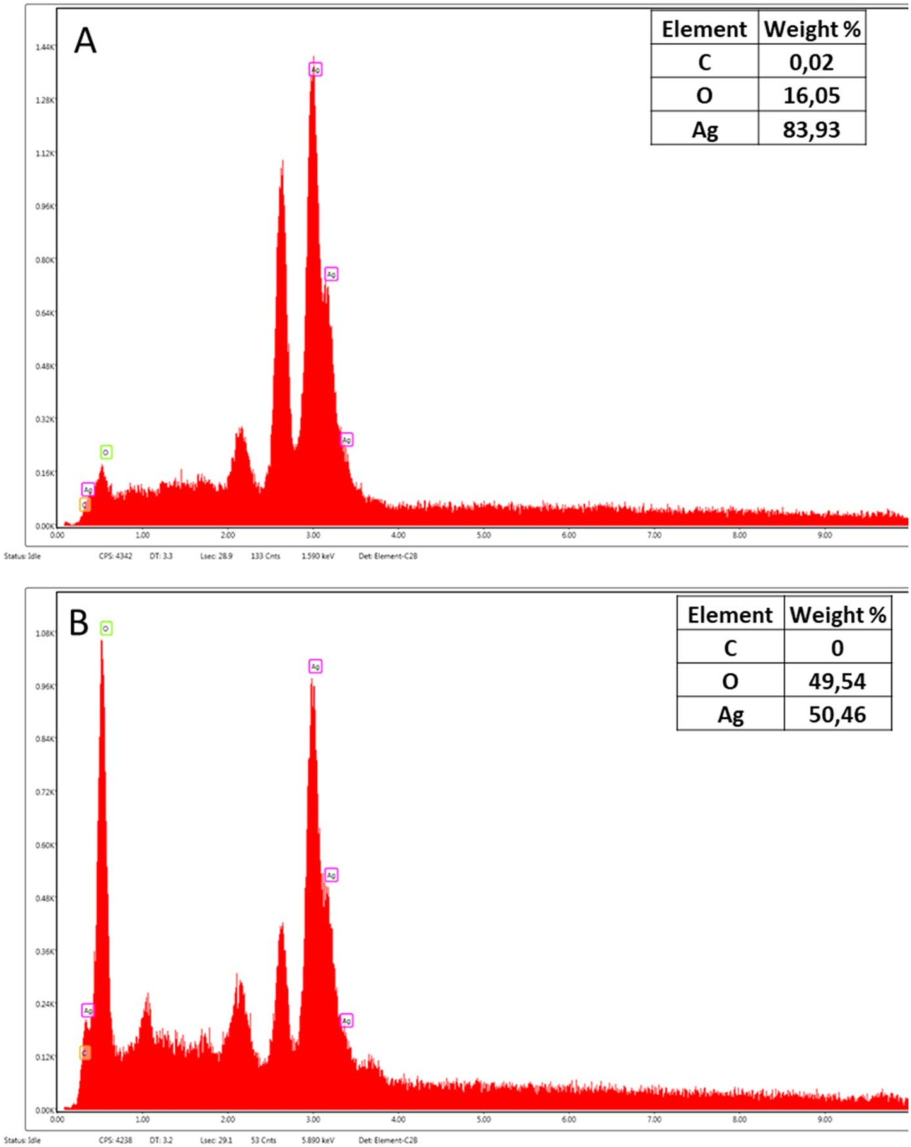

Fig. 6 EDS spectra of silver nanoparticles produced with 2.5 mM $AgNO_3$ and **A** 5% extract ratio; **B** 10% extract ratio

against bacteria, whereas the extract-mediated nanoparticles showed moderate activity. Therefore, it can be concluded that nanoparticles can enhance the activity of extracts.

Effect of *Rumex* sp. Silver Nanoparticles on Biofilm Formation

The antibiofilm activity of the *Rumex* sp. silver NPs was investigated against three isolates with moderate and strong biofilm-forming ability. First of all, the MIC value

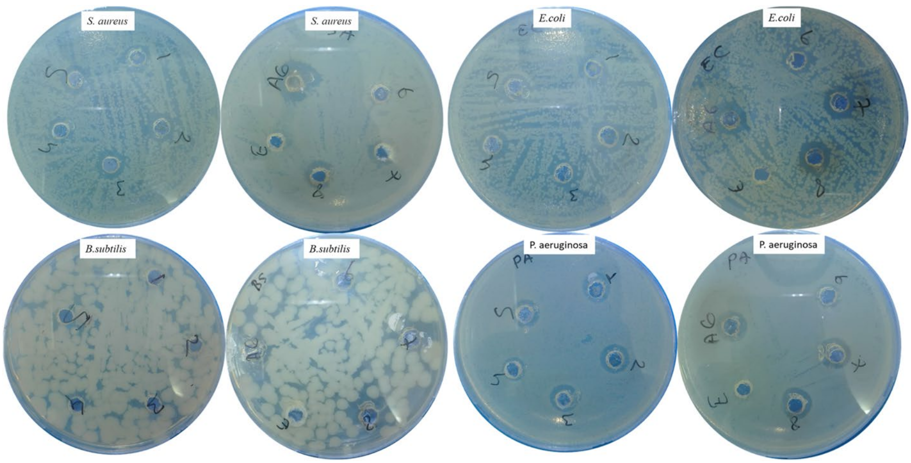

Fig. 7 Antimicrobial activity of synthesized nanoparticles against Gram-positive and Gram-negative bacteria

of the *Rumex* sp. silver NPs against these three isolates was found as 67.5 µg/mL. Nanoparticles at 1/2 MIC concentration was used to act as antibiofilm agents against the three strains (Table 3). At 125 µg/mL (1/2 MIC) concentration, the *Rumex* sp. NPs reduced the biofilm-forming capacity of *K. pneumoniae* by 2.66-fold, whereas that of *E. coli* was decreased by 1.25-fold. The quantitative biofilm-formation values of *A. baumannii* decreased by 2.66 with the 125 µg/mL *Rumex* sp. NP concentration. According to the data, the strains with moderate and strong biofilm-forming ability exhibited weak biofilm ability in the presence of 1/2 MIC concentrations of the *Rumex* sp. silver NPs.

Table 2 Antibacterial activity of synthesized silver nanoparticles against bacteria

Samples	Zone of inhibition mm			
	EC	SA	PA	BS
1	12	11	11	-
2	13	11	12	-
3	11	13	12	-
4	12	10	9	-
5	12	12	10	-
6	11	0	8	-
7	14	12	13	-
8	14	12	12	-
Extract	0	0	0	-
Ag 10 mM	15	17	13	-

EC Escherichia coli, *SA Staphylococcus aureus*, *PA Pseudomonas aeruginosa*, *BS Bacillus subtilis*

Table 3 Antibiofilm activity of *Rumex* sp. silver nanoparticles

Bacteria	Biofilm OD	125 µg/mL *Rumex* sp. nanoparticle
MDR *A. bumannii*	0.5492	0.2071
MDR *K. pneumoniae*	0.4835	0.1347
MDR *E. coli*	0.5855	0.4830
E. coli Dh5α (Control)	0.1272	-

Discussion

Considering the effect of extract ratio and $AgNO_3$ concentration on nanoparticle synthesis, it was observed that the amount (mg) of nanoparticles formed per unit volume increased with increasing both the extract ratio and $AgNO_3$ concentration. The maximum amount of silver nanoparticles was obtained in the experiments performed with 10 mM $AgNO_3$ and 10% leaf extract (Sample 5). It was also determined that the higher extract ratio increased the nanoparticle formation more than the $AgNO_3$ concentration did. As expected, similar outcomes were reported for the green synthesized silver nanoparticles from different plant extracts because the concentration of extracts increases the possibility to reduce the silver cations [15, 32].

A similar trend was observed in many studies in the literature, and it was interpreted that increasing the plant extract ratio accelerated the reducing of silver ions, preventing aggregation due to surface capping effects, and leading to the synthesis of smaller particles [1, 2, 13, 26]. Likewise, the same tendency was observed when EDS graphics and elemental composition were investigated. In the samples using the same silver concentration (2.5 mM) but different extract ratio (10%), it was determined that the weight percent of silver was lower at a higher extract ratio. In addition, due to the abundance of nanoparticle synthesis with a high extract ratio, it was seen from the SEM images that some nanoparticles were coagulated. The EDS spectra (Fig. 6) show the oxygen peaks along with silver, which indicates the produced nanoparticles are in the oxide form.

Silver nanoparticles were reported for being active against a varied range of microorganisms, and this feature was attributed to their multiple antimicrobial activity mechanisms [6, 10, 16, 24]. Recently, Singh et al. [27] reported that silver nanoparticles produced by green synthesis showed inhibition against *E. coli* and *S. aureus* as similarly observed in the current study. In the same study, the researchers also tested the combination of ampicillin and ciprofloxacin antibiotics with nanoparticles and showed that nanoparticle-antibiotic formulations were more effective than antibiotics alone [27]. In another study, Lara et al. showed that silver nanoparticles had bactericidal activity antibiotic resistant *E. coli*, *P. aeruginosa*, and *Streptococcus pyogenes*. This finding revealed that resistance proteins did not have any effects on silver nanoparticles and did not cause any change in the sensitivity of bacteria to nanoparticles [19]. It was also proved that the silver nanoparticle and antibiotic conjugates had bioactivity against multidrug-resistant and biofilm-forming microorganisms as well [31]. Another study demonstrated a synergistic relation between nanoparticles and antibiotics, thus, proving that antibiotics and nanoparticles were using different antibacterial mechanisms [29].

Biofilm formation plays a role in the development of many diseases such as otitis media, gingivitis, and lung infections, and it can cause bacteria to resist multiple antibiotics. The production and use of NPs are among the promising strategies to overcome drug-resistance mechanisms such as biofilm formation [24]. This study investigated the antibiofilm effect of *Rumex* sp. silver NPs against different antibiotic-resistant pathogenic bacteria with moderate and strong biofilm-forming ability. In the study conducted by Skora et al., the antibiofilm effect of silver NPs was investigated and different biofilm reduction percentages were determined. They found the best biofilm reduction for both *E. coli* (4.69-fold) isolate at 2 mg/mL concentration [28]. Martinez-Gutierrez et al. discussed the negative effects of AgNPs on many clinically important bacterial strains such as *A. baumannii* that are problematic in hospital treatment [20]. In a different study, more than 90% inhibition of live multidrug resistant (MDR) *A. baumannii* was seen at low AgNP doses, and this was reported to prevent the binding of *A. baumannii* on the human lung epithelial surface and subsequent biofilm formation [21, 33]. A study conducted in 2019 investigated the antibiofilm activity of tryptophan silver NPs on the biofilm formed by *E. coli* and*, K. pneumonia* under static conditions. The results showed that the NPs had an antibiofilm effect exceeding 50% on *E. coli* and *K. pneumonia* biofilm formation [9]. In the present study, *Rumex* sp. silver NPs reduced the biofilm-forming capacity of *A. baumannii* and *K. pneumonaie* strains by 2.66-fold and 3.25-fold, whereas it decreased that of *E. coli* by 1.25-fold. In this study, the investigated NPs were characterized by their high antibiofilm effect on bacterial biofilm formation. As shown in many different studies, AgNPs are seen as a promising antimicrobial agent to address the important public health problem of MDR isolates.

Conclusion

This study proposes an environmentally friendly, facile, and fast synthesis method for the production of silver nanoparticles. Within the scope of the study, the synthesis of silver nanoparticles at the size under 100 nm was successfully carried out by using aqueous extracts of *Rumex* sp. leaves as reducing agents. It was demonstrated that the size distributions and morphological structures of the synthesized silver particles were consistent. Structural and chemical characterizations were performed to reveal the involvement of leaf extract in the reduction of nanoparticles. The synthesized silver nanoparticles were also shown to pose antibacterial activity against Gram-positive and Gram-negative bacteria. Recently, nanotechnology offers products and methods that provide significant advantages in many areas. Due to their electrical, optical, and thermal properties, silver nanoparticles are not only widely used in the biomedical field, but also in different fields such as electronics, energy, and the environment.

Although the antibacterial properties of silver have been known for centuries, silver-based nanoparticles have the potential to be a promising method against increasing antibiotic resistance. Both the dimensional properties and physicochemical properties of silver nanoparticles enable them to inhibit or kill bacteria by different mechanisms. Due to these mechanisms, it is assumed that they can be a resourceful tool to combat bacteria with multi-drug resistance. Considering the rapid development of resistance to drugs worldwide, it is suggested that it can also be effective when conjugated with antibiotics due to their interactions with the bacterial membrane. The intensity in interest and application areas of silver nanoparticles has also increased the need for novel and effective synthesis methods. Green synthesis, which is a bottom-up production technique, is one of the most utilized techniques in recent times because of the elimination of toxic inputs and by-products. In this study, this technique was used in the synthesis of silver nanoparticles, including the optimization of the extract ratio and silver nitrate concentration. This study is one of a few studies in which *Rumex* sp. leaves are used as a reducing agent in the synthesis of silver nanoparticles, and it is expected that the findings will contribute significantly to the literature and future studies.

Author Contribution SA conducted the optimization, synthesis, and characterization experiments of the nanoparticle. SA wrote and edited the article. AÖD and GY conducted the antimicrobial and antibiofilm activity experiments, AÖD also contributed to the writing of the article. All the authors contributed to the study conception and design.

Data Availability Not applicable.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Competing Interests Not applicable.

References

1. Ahmed, T., & Ogulata, R. T. (2021). A review on silver nanoparticles - Green synthesis, antimicrobial action and application in textiles. *Journal of Natural Fibers, 00*, 1–22. https://doi.org/10.1080/15440478.2021.1964135
2. Ahsan, A., Farooq, M. A., Bajwa, A. A., Parveen, A. (2020). Green synthesis of silver nanoparticles using parthenium hysterophorus: Optimization, characterization and in vitro therapeutic evaluation. *Molecules*, *25*. https://doi.org/10.3390/molecules25153324
3. Akay, Ş., & Sefaoğlu, M. (2021). Yeşil sentez yoluyla mikrosistemlerde çinko oksit nanopartiküllerin üretimi ve karakterizasyonu. *Gümüşhane Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 11*, 315–324. https://doi.org/10.17714/gumusfenbil.819717
4. Alshameri, A. W., Owais, M., Altaf, I., & Farheen, S. (2022). Rumex nervosus mediated green synthesis of silver nanoparticles and evaluation of its in vitro antibacterial, and cytotoxic activity. *OpenNano, 8*, 100084. https://doi.org/10.1016/j.onano.2022.100084
5. Beykaya, M., & Çağlar, A. (2016). An Investigation on Synthesis of Silver-Nanoparticles (AgNP) and their Antimicrobial effectiveness by using Herbal Extracts. Afyon Kocatepe University Journal of Sciences and Engineering, 16(3), 631–641. https://doi.org/10.5578/fmbd.34220
6. Çalışkan, G., Mutaf, T., Öncel, S. Ş, & Elibol, M. (2019). Green synthesis of metal nanoparticles using microalga Galdieria sp. *IFMBE Proceedings, 73*, 219–224. https://doi.org/10.1007/978-3-030-17971-7_34
7. Ceylan, S., Cetin, S., Camadan, Y., Saral, O., Ozsen, O., & Tutus, A. (2019). Antibacterial and antioxidant activities of traditional medicinal plants from the Erzurum region of Turkey. *Irish Journal of Medical Science, 188*(4), 1303–1309. https://doi.org/10.1007/s11845-019-01993-x
8. Çimen, M., & Düzgün, A. Ö. (2020). Antibiotic induced biofilm formation of novel multidrug resistant Acinetobacter baumannii ST2121 clone. *Acta Microbiologica et Immunologica Hungarica, 68*(2), 80–86. https://doi.org/10.1556/030.2020.01240
9. Courrol, D. dos S., Lopes, C. R. B., Pereira, C. B. P., Franzolin, M. R., Silva, F. R. de O., & Courrol, L. C. (2019). Tryptophan Silver Nanoparticles Synthesized by Photoreduction Method: Characterization and Determination of Bactericidal and Anti-Biofilm Activities on Resistant and Susceptible Bacteria. *International Journal of Tryptophan Research, 12*. https://doi.org/10.1177/1178646919831677

10. Garibo, D., Borbón-Nuñez, H. A., de León, J. N. D., et al. (2020). Green synthesis of silver nanoparticles using Lysiloma acapulcensis exhibit high-antimicrobial activity. *Science and Reports, 10*, 12805. https://doi.org/10.1038/s41598-020-69606-7
11. Geremew, A., Carson, L., Woldesenbet, S. (2022). Biosynthesis of silver nanoparticles using extract of Rumex nepalensis for bactericidal effect against food-borne pathogens and antioxidant activity. *Frontiers in Molecular Biosciences*, *9*. https://doi.org/10.3389/fmolb.2022.991669
12. Hajipour, M. J., Fromm, K. M., Akbar Ashkarran, A., Jimenez de Aberasturi, D., Larramendi, I. R. de, Rojo, T., Mahmoudi, M. (2012). Antibacterial properties of nanoparticles. *Trends in Biotechnology, 30*(10), 499–511. https://doi.org/10.1016/j.tibtech.2012.06.004
13. Halawani, E. M. (2017). Rapid biosynthesis method and characterization of silver nanoparticles using Zizyphus spina christi leaf extract and their antibacterial efficacy in therapeutic application. *Journal of Biomaterials and Nanobiotechnology, 08*, 22–35. https://doi.org/10.4236/jbnb.2017.81002
14. He, X., Lu, F., Yuan, F., Jiang, D., Zhao, P., Zhu, J., Lu, G. (2015). Biofilm formation caused by clinical acinetobacter baumannii isolates is associated with overexpression of the adeFGH Efflux pump. *Antimicrobial Agents and Chemotherapy, 59*(8), 4817–4825. https://doi.org/10.1128/AAC.00877-15
15. Iravani, S., Zolfaghari, B. (2013). Green synthesis of silver nanoparticles using Pinus eldarica bark extract. *BioMed Research International*, *2013*. https://doi.org/10.1155/2013/639725
16. Jain, S., & Mehata, M. S. (2017). Medicinal plant leaf extract and pure flavonoid mediated green synthesis of silver nanoparticles and their enhanced antibacterial property. *Science and Reports, 7*, 1–13. https://doi.org/10.1038/s41598-017-15724-8
17. Jelinkova, P., Mazumdar, A., Sur, V. P., Kociova, S., Dolezelikova, K., Jimenez, A. M. J., Adam, V. (2019). Nanoparticle-drug conjugates treating bacterial infections. *Journal of Controlled Release, 307*(April), 166–185. https://doi.org/10.1016/j.jconrel.2019.06.013
18. Kotrange, H., Najda, A., Bains, A., Gruszecki, R., Chawla, P., & Tosif, M. M. (2021). Metal and metal oxide nanoparticle as a novel antibiotic carrier for the direct delivery of antibiotics. *International Journal of Molecular Sciences, 22*(17). https://doi.org/10.3390/ijms22179596
19. Lara, H. H., Ayala-Núñez, N. V., del Turrent, L. C. I., & Padilla, C. R. (2010). Bactericidal effect of silver nanoparticles against multidrug-resistant bacteria. *World Journal of Microbiology & Biotechnology, 26*, 615–621. https://doi.org/10.1007/s11274-009-0211-3
20. Martinez-Gutierrez, F., Boegli, L., Agostinho, A., Sánchez, E. M., Bach, H., Ruiz, F., & James, G. (2013). Anti-biofilm activity of silver nanoparticles against different microorganisms. Biofouling, 29(6), 651–660. https://doi.org/10.1080/08927014.2013.794225
21. McNeilly, O., Mann, R., Hamidian, M., & Gunawan, C. (2021). Emerging Concern for Silver Nanoparticle Resistance in Acinetobacter baumannii and Other Bacteria. *Frontiers in Microbiology, 12*, 652863. https://doi.org/10.3389/FMICB.2021.652863/BIBTEX
22. Murugan, K., Sanoopa, C. P., Madhiyazhagan, P., Dinesh, D., Subramaniam, J., Panneerselvam, C., Benelli, G. (2016). Rapid biosynthesis of silver nanoparticles using Crotalaria verrucosa leaves against the dengue vector Aedes aegypti: What happens around? An analysis of dragonfly predatory behaviour after exposure at ultra-low doses. *Natural Product Research, 30*(7), 826–833. https://doi.org/10.1080/14786419.2015.1074230
23. Ozturk, S., & Ozturk, A. (2007). Antibacterial activity of aqueous and methanol extracts of Rumex alpinus and Rumex caucasicus. *Pharmaceutical Biology, 45*(2), 83–87. https://doi.org/10.1080/13880200601105285
24. Pelgrift, R. Y., & Friedman, A. J. (2013). Nanotechnology as a therapeutic tool to combat microbial resistance. *Advanced Drug Delivery Reviews, 65*, 1803–1815. https://doi.org/10.1016/j.addr.2013.07.011
25. Rodríguez-León, E., Iñiguez-Palomares, R., Navarro, R. E., Herrera-Urbina, R., Tánori, J., Iñiguez-Palomares, C., & Maldonado, A. (2013). Synthesis of silver nanoparticles using reducing agents obtained from natural sources (Rumex hymenosepalus extracts). *Nanoscale Research Letters, 8*(1), 318. https://doi.org/10.1186/1556-276X-8-318
26. Rodríguez-León, E., Íñiguez-Palomares, R. A., Navarro, R. E., et al. (2018). Silver nanoparticles synthesized with Rumex hymenosepalus extracts: Effective broad-spectrum microbicidal agents and cytotoxicity study. *Artificial Cells, Nanomedicine, and Biotechnology, 46*, 1194–1206. https://doi.org/10.1080/21691401.2017.1366332
27. Singh, M., Renu, Kumar, V., et al. (2021). Biomimetic synthesis of silver nanoparticles from aqueous extract of Saraca indica and its profound antibacterial activity. *Biointerface Research in Applied Chemistry, 11*, 8110–8120. https://doi.org/10.33263/BRIAC111.81108120
28. Skóra, B., Krajewska, U., Nowak, A., Dziedzic, A., Barylyak, A., & Kus-Liśkiewicz, M. (2021). Noncytotoxic silver nanoparticles as a new antimicrobial strategy. *Scientific Reports, 11*(1), 1–13. https://doi.org/10.1038/s41598-021-92812-w

29. Slavin, Y. N., Asnis, J., Häfeli, U. O., & Bach, H. (2017). Metal nanoparticles: Understanding the mechanisms behind antibacterial activity. *Journal of Nanobiotechnology, 15*, 1–20. https://doi.org/10.1186/s12951-017-0308-z
30. Thirumurugan, G., Seshagiri Rao, J. V. L. N., & Dhanaraju, M. D. (2016). Elucidating pharmacodynamic interaction of silver nanoparticle - Topical deliverable antibiotics. *Scientific Reports, 6*(April), 1–11. https://doi.org/10.1038/srep29982
31. Thomas, R., Jishma, P., Snigdha, S., et al. (2020). Enhanced antimicrobial efficacy of biosynthesized silver nanoparticle based antibiotic conjugates. *Inorganic Chemistry Communications, 117*, 107978. https://doi.org/10.1016/j.inoche.2020.107978
32. Umoren, S. A., Obot, I. B., & Gasem, Z. M. (2014). Green synthesis and characterization of silver nanoparticles using red apple (Malus domestica) fruit extract at room temperature. *Journal of Materials and Environmental Science, 5*, 907–914.
33. Wintachai, P., Paosen, S., Yupanqui, C. T., & Voravuthikunchai, S. P. (2019). Silver nanoparticles synthesized with Eucalyptus critriodora ethanol leaf extract stimulate antibacterial activity against clinically multidrug-resistant Acinetobacter baumannii isolated from pneumonia patients. *Microbial pathogenesis, 126*, 245–257. https://doi.org/10.1016/J.MICPATH.2018.11.018