

Silver nanoparticles produced from *Agropyron repens*: A green synthesis approach and antimicrobial analysis

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Abstract

The plant Agropyron repens is invasively distributed both in natural grassland areas and in agricultural areas. It contains valuable metabolites such as carbohydrates, phenolic compounds, flavonoids, saponins, essential oils, and minerals. A. repens plant extract has the potential as an alternative source for nanoparticle synthesis. The total phenolic and carbohydrate contents of the aqueous extract were analyzed using spectrophotometric methods. Silver nanoparticles (AgNPs) were synthesized at room temperature using the aqueous extract of A. repens without any chemical stabilizer or reducing agent. The formation of AgNPs was confirmed by the observed color change. UV-Vis spectroscopy, dynamic light scattering (DLS), Fourier transform infrared spectroscopy, and X-ray diffraction were used for characterization. An absorption was obtained in the UV-Vis spectrum. In DLS measurements, the size of AgNPs was determined to be approximately 75 nm. The obtained AgNPs and fabrics treated with these nanoparticles showed antibacterial activity against both gram positive (Staphylococcus aureus) and gram negative (Escherichia coli) bacteria. AgNPs and fabric samples exhibited antifungal activity against Candida albicans. In addition, the antioxidant activities of aqueous extracts and AgNPs were evaluated. This study highlights that A. repens can be used in the production of AgNPs by green synthesis methods.

Introduction

In the last few years, advances in bio-based technologies have led to a convergence between nanotechnology and green chemistry (Fawcett et al., 2017; Dutta & Das, 2021). Nanotechnology, which is the development of familiar materials with a range of different properties, has seen successful and rapid growth (Carmona et al., 2017). An important aspect of nanotechnology is directly controlling their morphology of the particles and the size during the synthesis and formation of nanoscale materials. Nanotechnology is a collection of nanostructures, the dimensions of which

range from 0 to 3-Dimensions, where the scale of the matter is between 1 nm and 100 nm (Deivanathan & Prakash, 2022). It can be particles, plates, tubes, sheets, wires and many other geometric shapes. Numerous studies have shown that materials at the nanometer scale have physical, chemical, biological, electronic, thermal optical and mechanical properties that significantly differ from their bulk counterparts. Broadly speaking, nanomaterials can be classified as organic or inorganic. While inorganic nanomaterials include metals (silver, gold, and platinum), semiconductors (zinc oxide)

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and titanium dioxide), magnetic materials (iron oxide), and organic nanomaterials are carbon-based (<u>Fawcett</u> et al., 2017; Saleh, 2020).

The production of nanoparticles could be broadly characterized by two approaches. The top-down approach is the first, which involves subjecting a material to size reduction through chemical or physical processes. The bottom-up approach is the second and is the creation of nanoparticles through the assembly of atoms, molecules, monomers, or smaller particles (Khanna et al., 2019). Unfortunately, most of the physical and chemical processes used in both approaches have some disadvantages, such as being technically complex, low rate of conversion of materials, expensive, and high demand for energy. In addition, most of these processes use hazardous chemicals. These include organic solvents, reducing agents, and nonbiodegradable stabilizers. For this environmentally friendly processes based on green nanotechnology to produce nanoparticles have attracted a great deal of interest (Fawcett et al., 2017; Hano & Abbasi, 2021).

Currently, green synthesis has been used to produce nanoparticles in the field of nanomaterials (Carmona et al., 2017). This alternative approach focuses on biological entities. Biosynthesis via unicellular or multicellular biological entities like bacteria, actinomycetes, viruses, yeasts, fungi, algae, and plants, offers alternative environmentally friendly approaches for nanoparticle production. Each of the biological units possesses active compounds that can behave as reducing/stabilizing agents to nanoparticles synthesis of various shapes, sizes, compositions or physicochemical properties (Fawcett et al., 2017; Ramakrishna et al., 2016). Plant extracts can be a reduction agent and also a stabilizing agent for the system (Carmona et al., 2017). Different phytomolecules present in plants, such as polyphenols, flavonoids, sugars, and proteins, function in the nanoparticle formation (Hawar et al., 2022). In a one-step green synthesis process, biomolecules in plant extracts could reduce metal ions to the nanoparticles. Biogenic reduction of metal ions is very rapid. It can be performed at room temperature or pressure and is scalable with simplicity (Mittal et al., 2013).

Besides medical applications, metal nanoparticles have a wide range of applications in electronics, cosmetics, optics, coatings, space industries, sensing devices, food packaging, bioremediation, therapeutics, environmental health, light emitters, mechanics, chemical industries, non-linear optical devices and photo-electrochemical applications (Khanna et al., 2019). Through their biological functionalization, greensynthesized nanoparticles have important medical applications, particularly in terms of antimicrobial activity. In addition to these applications, there are also various studies aiming to produce fabrics with different performances by integrating nanoparticles into textile materials (El-Rafie et al., 2013).

Among various metallic nanoparticles, silver nanoparticles (AgNPs) have attracted considerable interest because of their non-toxicity, hydrophilicity, structure, ease of preparation, environmental adaptability (Deivanathan & Prakash, 2022). Historically, the antimicrobial properties of silver have attracted great attention. Large-scale production **AgNPs** with unique physicochemical antimicrobial properties has become possible due to advances in manufacturing processes. The antimicrobial properties of AgNPs against various pathogenic microorganisms have led to their inclusion in a wide range of medical and pharmaceutical protocols. Not fully understood are the mechanisms in connection with their antimicrobial properties. Therefore, significant membrane damage and biological disruption are believed to result from the interaction between microbial cell membranes and AgNPs (Fawcett et al., 2017; Roy et al., 2019).

Agropyron repens is an invasive plant species that ranges from temperate Europe to Central Asia and even Africa. A. repens, also named as couch grass, especially in agricultural areas, has a high potential to be utilized in the field of biotechnology (Al-Snafi., 2015). It has traditionally been used as a sedative and diuretic to relieve spasms and pain in the urinary tract (Deveci et al., 2020). The plant contains carbohydrates, mucins, pectin, tritisin, cyanogenic glycosides, phenolic compounds, flavonoids, saponins, essential oils, volatile oils, vanillin glucoside, and minerals such as iron and silica. It has anti-inflammatory, hypolipidemic, hypoglycemic and diuretic effects (Bortolami et al., 2022).

In this study, a cheap, biological, and environmentally friendly green synthesis method was employed to synthesize AgNPs from AgNO₃ precursor using aqueous *A. repens* extract. The bioactive components present in the *A. repens* extract were successful in the reduction reaction. Fourier transform infrared spectroscopy (FTIR), dynamic light scattering (DLS), Ultraviolet-visible spectroscopy (UV-Vis) and X-ray diffraction (XRD) were used to characterize the synthesized AgNPs. The antioxidant and antimicrobial activities of AgNPs were also evaluated.

Materials and Methods

<u>Preparation of the aqueous extract of Agropyron</u> <u>repens</u>

The plant was used for the production of AgNPs due to its cost-effectiveness and easy availability. *A. repens* was purchased from Arifoglu company. The plant was weighed (10 g), and 100 mL of deionised water was added. Extraction was carried out in a shaking water bath (Wisd) at 80°C for 1 hour. The solid-liquid was separated by coarse filtration. This extract was stored at 4°C until needed for the synthesis of AgNPs.

Extract analyses of Agropyron repens

In order to analyze the content of the obtained extracts spectrophotometrically, the Folin-Ciocalteu and the Dubois methods were performed.

Total phenolic matter determination

The Folin-Ciocalteu method was used to determine the total phenolic content. The standard used was gallic acid (Sigma-Aldrich). 200 μL of 0.2 N Folin-Ciocalteu solution (Sigma-Aldrich) was added to 100 μL of the sample and mixed. Then, 2 mL of distilled water and 1 mL of a 6% solution of sodium carbonate (Na₂CO₃) (Merck) were added and vortexed. The mixture was kept at room temperature for 2 hours in the dark. The spectrophotometer was used to read the absorbance of the color formed at 765 nm. For gallic acid standards, the same procedure was used. The calibration curve was plotted, and the results were obtained as gallic acid equivalent (GAE) (Miceli et al., 2009).

Total carbohydrate content determination

The total carbohydrate content of the extracts was measured by the Dubois method. As a standard solution, glucose was prepared. The first step was to take 0.5 mL of the extract and transfer it to a glass tube. For the blank, water was used. 0.5 mL of 5% phenol solution was added to the tubes. Then 2.5 mL of concentrated sulphuric acid (Merck) was added. The sample was vortexed. The absorbance against the blank in a spectrophotometer (EasyPlus, Mettler Toledo) was read after 15 minutes in a water bath. The results were calculated according to the glucose standard (Dubois, 1956).

Green synthesis of silver nanoparticles

From the results of preliminary experiments, 10 mL of the *A. repens* extract was added dropwise to 90 mL (1:9) 1.89 mM AgNO₃ solution under neutral pH and at room temperature. After 2 hours of incubation, there was a visible color change in the solution containing AgNPs. No additional chemicals were required to synthesize and stabilize the AgNPs produced by this process because of the biometabolites in the *A. repens*. The solution containing the nanoparticles was centrifuged (Nüve, NF400) at 9000 rpm for 15 minutes to allow the AgNPs to separate from the remaining solution after the synthesis of AgNPs. The nanoparticles obtained were then dried at 60 °C for 48 hours and were obtained in the form of a powder.

Characterization of silver nanoparticles

UV-Visible spectroscopy, dynamic light scattering, Fourier transform infrared, and X-ray diffraction methods were used to characterize the AgNPs.

UV-Visible Spectroscopy

Nanoparticle formation was confirmed using UV-Vis spectroscopy. For this reason, a UV-Vis spectrophotometer was scanned in the wavelength range of 190-900 nm and the absorbance value at which the maximum peak of AgNPs was determined.

Dynamic Light Scattering (DLS)

The DLS was used to measure the average size of the AgNPs (Malvern Zetasizer Analyzer). The size distribution of particles in colloidal suspension has also been characterized. Measurements were repeated three times for the samples.

Fourier Transform Infrared (FTIR)

FTIR spectroscopy is used to characterize the surface chemistry and the bonds between functional groups. Organic functional groups such as hydroxyl, carbonyl, and other surface chemical residues bound to the nanoparticle surface are detected. The dry powder material was for analysis. The structural characteristics of the AgNPs were investigated using FTIR spectroscopy (Thermo Scientific, Nicolet, IS20, USA) in the wave number range of 500 cm⁻¹ to 4000 cm⁻¹.

X-ray diffractometer (XRD)

The phase composition and quality of the nanoparticles were checked by XRD (X-ray diffractometer; PANanalytical Empyrean, U.K.) using Cu-K α radiation between $2\theta=10\text{-}80^\circ$ at a scan rate of 2° /min. The size of the synthesized AgNPs was also evaluated using the Debye-Scherrer equation. D is the average size of AgNPs, K is constant (K = 0.94), λ is the wavelength of X-ray (0.1546 nm), θ is diffraction angle (in degrees) and β is the width of the maximum peak at half of the height (Bagherzade et al., 2017).

$$D = \frac{K.\lambda}{\beta.\cos\theta}$$

Antimicrobial activity of silver nanoparticles

Antimicrobial activity was determined by antibacterial analysis using the Kirby-Bauer disc diffusion technique and antifungal analysis using the agar well diffusion technique.

Antibacterial activity

Antibacterial tests were conducted on the grampositive bacterium Staphylococcus aureus (ATCC 6538) and the gram-negative bacterium Escherichia coli (ATCC-25922) using the Kirby-Bauer disk diffusion technique (Hudzicki, 2009). The bacterial suspensions were diluted to an optical density of 0.6 at a wavelength of 600 nm and inoculated into Mueller-Hinton agar (Merck) by the spread-plate method, as calculated. After inoculation, the samples (plant extract, AgNPs, AgNO3, and control samples) diluted at different concentrations were placed on 25 µL volume 6 mm diameter sterile antibacterial susceptibility (Oxoid) disks and cotton fabrics. After that, samples were placed on agar. Analyses were also carried out on cotton fabrics to test the antibacterial performance of the samples. Samples of fabric known to be 100% cotton were sterilized by

cutting them into 1x1 cm pieces and immersing the sterilized fabric in the samples for 30 minutes. After incubating the petri dishes at 37°C for 24 hours, the antibacterial inhibition zones were evaluated by measuring the diameters of the zones around the discs and fabrics. Gentamicin antibiotic was used as a positive control and distilled water was used as a negative control during antibacterial activity tests (Biemer, 1973).

Antifungal activity

The efficacy of the samples against *Candida albicans* was tested by the agar well diffusion method (EUCAST, 2020). In this context, PDA (Merck) containing *C. albicans*, the final concentration of which was adjusted to 10^5 in the medium, was poured into the petri dishes and the wells were opened with a hollow sterile glass rod with a diameter of 5 mm. $100 \, \mu L$ of sample was added to the wells and the petri dishes were incubated at 37 °C for 24 hours (EUCAST, 2020). Inhibition zones of the samples against *C. albicans* were determined by measuring the zone diameters formed at the end of incubation. The antifungal agent fluconazole was used as a positive control during the experiments.

Determination of antioxidant activity

The antioxidant activity was defined according to the DPPH (2,2-Diphenyl-1-picrylhydrazyl) (Tokyo Chemical Industry Co. Ltd.) method. $50~\mu L$ of the sample extract was transferred to glass test tubes and $150~\mu L$ of methanol solution (Merck) was added. This was followed by the addition of 3.8~mL of DPPH solution. For half an hour the tubes were vortexed and kept in the dark. The absorbance values were read in a spectrophotometer at a wavelength of 515~nm. As a positive control, methanol was used. The results obtained in this method were expressed as % inhibition. The same procedure for BHA (butylated hydroxyanisole) (Sigma Aldrich) was carried out for comparison (Seyrekoğlu & Temiz, 2020).

$$Inhibition\% = \frac{Absorbance_{positive\ control} - Absorbance_{sample}}{Absorbance_{positive\ control}} \times 100$$

Results and Discussion

Analysis of aqueous extract

Nanoparticles can be synthesized biometabolites such as phenolics, amides, amines, proteins, carbohydrates, pigments, or alkaloids through clean, environmentally friendly, easy preparation and biogenic ways. Therefore, it is also important to investigate the contents of the plant material used in the green synthesis method. Carbohydrates and phenolic compounds, which are present in A. repens, may be involved in reducing and stabilizing silver ions. For this reason, the total carbohydrate contents, the total amount of phenolic matter, and the antioxidant activity of the aqueous A. repens extract were determined using spectrophotometric methods. The amount of total phenolics, the amount of total carbohydrates and the antioxidant activity (DPPH) were measured as 3.6 μ g GAE/mL, 354 μ g/mL, and 18.90%, respectively (<u>Table 1</u>). In addition to the investigation of total carbohydrate content as a reducing agent, antioxidant activity is mainly due to phenolic content (<u>Wojdyło et al., 2007</u>).

Table 1. Agropyron repens extract analysis

-		Total phenolic matter (μg GAE/mL)	Total carbohydrate content (µg/mL)	Antioxidant Activity (%)
	Agropyron repens extract	3.6	354	18.90

The green synthesis of AgNPs was carried out by adding the extract of A. repens to aqueous silver nitrate solutions and reducing Ag^{2+} ions to Ag^0 . The color change of the solution in AgNPs formation is considered as the primary analysis due to particle excitation of surface plasmon vibrations (Hawar et al., 2022). As the negative control (aqueous silver nitrate solution only) was colorless, it was also compared with visual observation (Figure 1).



Figure 1. Synthesis steps and color change of AgNPs.

Characterization of silver nanoparticles

The size of the synthesized AgNPs is 74.50 nm according to the DLS size distribution histogram, while the polydispersity index (PDI) value is 0.293 (Figure 2). In a study, Deivanathan and Prakash synthesized AgNPs from aqueous leaf extract of Guettarda speciosa and found that the average size was 157.6 nm according to DLS of hydrodynamic measurement (Deivanathan & Prakash, 2022). This study shows that AgNPs were successfully synthesized with a size of approximately 75 nm when evaluated at the nanometer scale. In addition, repeated DLS measurements show consistencies in nanoparticle size. The PDI value is used to describe the dispersion and agglomeration of nanoparticles in the sample. A low PDI value indicates that the AgNPs distribution in the sample is monodisperse (Mudalige et al., 2018).

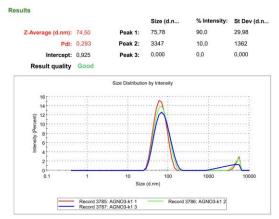


Figure 2. The DLS and PDI values of AgNPs.

In the UV-Vis spectrum, the surface plasmon resonance of AgNPs occurred near 320 nm and the reduction of silver nitrate to AgNPs is indicated by this peak (Figure 3). Absorbance peak formation in the range of 320 nm in UV-Vis spectrophotometer scanning indicates that the synthesized nanoparticles show a monodisperse structure and uniform distribution. The formation of the absorbance peak in the range of 300-350 nm indicates that the nanoparticles in the structure show a uniform distribution. The distribution structure obtained as a result of UV-Vis analysis is supported by the PDI value determined in the DLS analysis (Saeb et al., 2014).

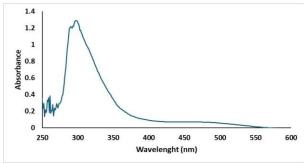


Figure 3. The UV-Vis spectrum of AgNPs.

FTIR analysis confirmed the role of the extract as a reducing and capping agent and the presence of some functional groups. The FTIR spectrum of AgNPs is shown in Figure 4. Functional groups at 3370, 2914, 1662, 1378, and 1037 cm⁻¹ are shown in the AgNPs. The peaks at 3370 cm⁻¹ correspond to OH stretching vibrations of carboxylic groups and phenols from the extract, as well as N-H stretching vibrations of NH₂ groups, 2914 cm⁻¹ is attributed to C-H stretching vibrations (presence of the polyphenolic compound), 1662 cm⁻¹ indicates amides with C=O stretching vibrations, 1378 cm⁻¹ depicts alkanes with C-H bending and 1037 cm⁻¹ corresponds to alcohols with C-O stretching. These clear peaks are consistent with the literature. Therefore, the compounds extracted from the A. repens plant have a key role in reducing, stabilizing and capping functions, according to the FTIR results. The presence of some functional groups capping the AgNPs is confirmed by this measurement (Carmona et al., 2017; Salayova et al., 2021; Sathishkumar et al., 2019; Widatalla et al., 2022).

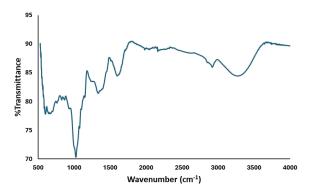


Figure 4. The FTIR spectra of AgNPs.

As shown in Figure 5, the XRD pattern of synthesized AgNPs shows the four strong peaks at 38.21°, 44.33°, 64.55° and 76.81° corresponding to (111), (200), (220), and (311) planes, respectively, and these crystallographic planes are Face Centered Cubic (FCC) (Deivanathan & Prakash, 2022). Using Scherrer's equation, the average crystallite size of the synthesized nanoparticle was calculated as 54.87 nm.

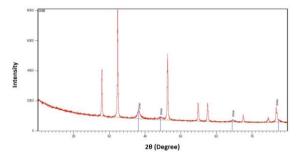


Figure 5. The XRD pattern of AgNPs.

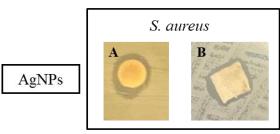
Biological activity of silver nanoparticles

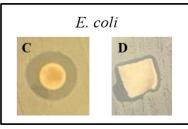
The antioxidant activity of the A. repens extract and produced AgNPs was also evaluated using the DPPH method (Table 2). These values have been compared with those of BHA. DPPH is a more stable and betterknown free radical, which is the result of the reduction of hydrogen or electrons from donors (Bhakya et al., 2016). The ability of the AgNPs to reduce DPPH was assessed by observing the color change, and the control did not show any change in color. According to this method, the concentrations of A. repens extract, AgNPs and BHA were 18.90%, 8.17%, and 98.02%, respectively. It is thought that the DPPH activity of AgNPs may increase in a dose-dependent manner. The functional groups attached to the AgNPs, which originate from the A. repens extract, are responsible for the antioxidant potential of the AgNPs.

Table 2. Antioxidant activities of extract, AgNPs and BHA

	Antioxidant activity (%)		
Agropyron repens-extract	18.90		
Agropyron repens-AgNPs	8.17		
BHA (butylated hydroxyanisole)	98.02		

Based on the antibacterial analyses, while $AgNO_3$ and AgNPs showed activity against both *S. aureus* and *E. coli, A. repens* extract showed no antibacterial activity. Within the scope of this study, AgNPs synthesized by





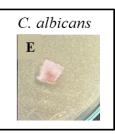


Figure 6. Antimicrobial effect of AgNPs, A) S. aureus disc diffusion method, B) S. aureus fabric sample, C) E. coli disc diffusion method, D) E. coli fabric sample, E) C. albicans fabric sample.

using the extract of *A. repens* showed an inhibition zone of 8.33 mm against *S. aureus* and 9.67 mm against *E. coli* at a 2 mg/mL concentration. On the other hand, zone diameters of 9.5 mm against *S. aureus* and 10.17 mm against *E. coli* were observed in trials using AgNO₃. Inhibition zones with a diameter of 16 mm were observed against the antibiotic gentamicin *S. aureus* and *E. coli* used as a positive control in the study. In this context, it can be said that the synthesis of AgNPs using the extract increases the antibacterial activity of the extract but reduces the effect of AgNO₃.

When the fabric samples were analyzed, it was observed that the inhibition zone of A. repens extract and AgNO₃ was 12 mm against S. aureus and 14 mm against E. coli. While AgNPs coated fabrics showed a 16 mm inhibition zone against S. aureus and E. coli, gentamicin added fabrics showed a 37 mm inhibition zone against S. aureus and a 38 mm inhibition zone against E. coli. The observation of inhibition zones at higher rates in the fabric samples can be considered as an indication that the samples are better adhered to the fabric surface. The antibacterial effect of the extract was enhanced by AgNPs synthesis. Detailed information on antibacterial analyses of fabric samples is given in Table 3 and Figure 6. AgNPs synthesized biogenically by Deivanathan and Prakash showed a zone diameter of 5 mm for E. coli and 6 mm for S. aureus in antibacterial tests. In addition, a 3 mm inhibition diameter was obtained with Guettarda speciosa extract used in the synthesis. The positive control is 7 mm. In antifungal trials, a 2 mm zone diameter was measured against Aspergillus flavus in the nanoparticle and a 1 mm zone diameter in the extract. The positive control is 8 mm (Deivanathan & Prakash, 2022).

In the antifungal analysis performed against *C. albicans* within the scope of the study, no visible inhibition zones were observed by the agar well diffusion method. When the fabric samples were examined, it was observed that inhibition zones with a diameter of 15 mm were formed in AgNPs coated fabric samples, while no inhibition zones were formed in extract and AgNO₃ coated fabric samples. Fabric

samples coated with the antifungal agent fluconazole, which was used as a positive control, formed inhibition zones with a diameter of 17 mm shown in Figure 6. As a result of the antifungal analyses, AgNPs synthesized by using the extract of A. repens formed inhibition zones, indicating that AgNPs synthesis increases the antifungal effect. The fact that the inhibition zones of AgNPs coated fabric samples are very close to the inhibition zones of fluconazole coated fabric samples can be considered as an indication that the synthesis of AgNPs using A. repens extract will be an antifungal effective component. In a study, Hawar et al., conducted the synthesized AgNPs from Alhagi graecorum leaf extract and studied the antifungal activity against Candida albicans, Candida glabrata, Candida parapsilosis, Candida tropicales, and Candida krusei with the well diffusion method. The inhibition zone that was calculated was ranging from 14- 27 mm for different concentrations (Hawar et al., 2022).

In this study, an efficient bioproducer for the synthesis of AgNPs has been demonstrated in *A. repens*. Secondary metabolites clearly showed the reduction of Ag⁺ ions to Ag⁰. It is important to search for the contents of biological resources in the green synthesis of nanoparticles. By optimizing the extraction conditions, higher levels of phenolics and sugars can be obtained. Also, the antioxidant and antimicrobial activities of the AgNPs obtained can be re-evaluated by optimizing their production under a variety of conditions. Antimicrobial activities in textile applications can be improved and enriched in future studies.

Conclusion

Silver nanoparticles could be successfully biosynthesized without the need for external chemicals, stabilizers or reducing agents in a fast, environmentally friendly, cost-effective, and efficient method. A onestep green synthesis of stable AgNPs using *A. repens* extract at room temperature was reported in this work. The first indication of nanoparticle formation was observed through a color change. The synthesized

Table 3. Antimicrobial activities of fabric samples

		Inhibition zones (mm)				
	Microorganism	Agropyron repens extract	AgNPs	AgNO₃	Antimicrobial Agent	
Antimicrobial activity	S. aureus	12	16	12	37	
Antimicrobial activity	E. coli	14	16	14	38	
Antifungal activity	C. albicans	-	15	-	17	

AgNPs were characterized using UV-Vis, DLS, FTIR, and XRD. These results indicated that the extract of *A. repens* has the potential for nanoparticle production. The antioxidant and antimicrobial activities of AgNPs were also evaluated. AgNPs showed inhibition zones of 8.33 mm against *S. aureus* and 9.67 mm against *E. coli*, while the inhibition zone of the AgNPs coated fabrics against *C. albicans* was 15 mm. When different metal nanoparticles are examined, AgNPs draw attention to their antimicrobial effects. It is very important to use different wastes or inert plant extracts in the green synthesis of AgNPs and thus, synthesize different nanoparticle structures with the recovery of components.

Author Contributions

Conceptualization: AI, NO, ZG, ME, Data Curation: AI, NO, ZG, ME, Formal Analysis: AI, NO, ZG, ME, Funding Acquisition: ME, Investigation: AI, NO, ZG, ME, Methodology: AI, NO, ZG, ME, Project Administration: AI, NO, ZG, ME, Resources: AI, NO, ZG, ME, Supervision: ME, Visualization: AI, NO, ZG, ME, Writing -original draft: AI, NO, ZG, ME, Writing -review and editing: AI, NO, ZG, ME.

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