Green synthesis of silver nanoparticles from Polygonum aviculare L. extract, evaluation of antibacterial, antioxidant activity

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Abstract

Introduction & Objective: Recently, the utilization of herbals for the biosynthesis of nanoparticles has been noticed in their properties such as environmental compatibility, simplicity, and economics. Numerous medicinal plants possess the ability to synthesize nanoparticles that have applications in medicine, biosciences, and the pharmaceutical industry. The primary objective of this research is to propose an environmentally friendly approach for synthesizing silver nanoparticles (AgNPs) for use in drug delivery, medicine, industry. **Methods:** First, the *polygonum aviculare L.* extract was prepared by maceration and synthesized by 2 *mM* $AgNO_3$ solution using the green method. After changing the color of the extract, confirmation of the formation of nanoparticles by UV-Vis spectroscopy, and Morphological investigation by TEM, FE-SEM, XRD and FT-IR to examine functional groups in nanoparticles and investigation of antioxidant and antibacterial activities of nanoparticles by DPPH assay and Disk Diffusion method. **Results:** The emergence of an absorption peak between the wavelengths of 400-450 nm on the UV-Vis spectrophotometer indicates the synthesis of silver nanoparticles. The shape of spherical and rod particles, their average size is about 25-70 *nm*. The free radical scavenging activity was calculated as $IC_{50} = 15.63$ *mg/L*. The synthesized silver nanoparticles had significant antimicrobial activity. **Conclusion:** *polygonum aviculare L.* extract can reduce Ag^+ ions to silver nanoparticles. In addition, synthesized silver nanoparticles have good antioxidant and antimicrobial properties.

Green synthesis of silver nanoparticles from *Polygonum aviculare L.*extract, evaluation of antibacterial, antioxidant activity

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Methods: First, the *polygonum aviculare L.* extract was prepared by maceration and synthesized by 2 *mM* $AgNO_3$ solution using the green method. After changing the color of the extract, confirmation of the formation of nanoparticles by UV-Vis spectroscopy, and Morphological investigation by TEM, FE-SEM, XRD and FT-IR to examine functional groups in nanoparticles and investigation of antioxidant and antibacterial activities of nanoparticles by DPPH assay and Disk Diffusion method.

Results: The emergence of an absorption peak between the wavelengths of 400-450 nm on the UV-Vis spectrophotometer indicates the synthesis of silver nanoparticles. The shape of spherical and rod particles, their average size is about 25-70 nm . The free radical scavenging activity was calculated as $\mathrm{IC}_{50} = 15.63 mg/L$. The synthesized silver nanoparticles had significant antimicrobial activity.

Conclusion: *polygonum aviculare L.* extract can reduce Ag^+ ions to silver nanoparticles. In addition, synthesized silver nanoparticles have good antioxidant and antimicrobial properties.

Keywords: Silver nanoparticles, Green synthesis, Antibacterial, Antioxidant, *polygonum aviculare L.*

Highlights:

- Silver nanoparticles were synthesized by the green method.
- Morphologically investigated by XRD, FESEM, TEM, and FT-IR, and their results showed spherical nanoparticles with a particle size of 25-70nm .
- Silver nanoparticles had significant antioxidant properties.
- Silver nanoparticles had more antibacterial properties against E. coli than S. aureus.

Introduction

Today, nanotechnology is one of the most widely used fields of science and nanotechnology in the world (1). In addition, this interdisciplinary field is applied in the field of physics, electronic materials, biology, agriculture, chemistry and medicine, which uses these scientific fields to control matter at the molecular scale (2). The utilization of medicinal plants for the synthesis of nanoparticles is a relatively uncomplicated and cost-effective approach (3,4) and one of the advantages of this method is non-toxicity (5). The green synthesis method of metal ions using plant compounds is usually a one-step reaction that transforms into nanoparticles without the need for stabilizing agents. Biologically active substances and compounds in plant extracts such as flavonoids and other effective water-soluble active metabolites can cause the reduction of metal ions and the formation of nanoparticles at room temperature (6). generally, green chemistry is a research or innovation method that includes a practical part for the design, development, and efficient production of products that can minimize hazardous substances for health (7). Nanoparticles have special features like size, shape, and morphology, and for this reason, they are widely used in human life. Among them, metal nanoparticles such as silver, gold, platinum, and palladium are widely used by physicians and are healthy (8). Among the various nanoparticles synthesized, AgNPs are recognized as one of the most extensively employed nanotechnologies in disease treatment. A significant amount of research has been conducted on their potential anticancer (9), antibacterial (10), antifungal (11), and antiviral (12) effects. However, silver alone may have limited properties or play a minor role (13). Hera et al. discovered that Ag can be produced from alfalfa biomass aqueous solution that is pH-dependent (14). biosynthesis of silver nanoparticles by plants such as aqueous and alcoholic extracts of Aloe vera (15), the leaf extract of South African artemisia (Afra Artemisia) (16), aqueous extract of aerial part of Corniculum Glaucium (8), plant felt leaf extract of Abutilon Indicum (L.) Sweet (17) And many other plants have been done. Many reports exist about the use of synthesized silver nanoparticles and their antibacterial properties. For example, the results of the studies of Aziziyan Sharmeh et al. Nanoparticles are synthesized using an aqueous extract from the Sambucus nigra plant (18). Heidarzadeh et al. green synthesis of silver nanoparticles Extracted by Citrus aurantium extract (19) and in the study of Sheikh Rafi et al., nanoparticles were synthesized by Origanum Majorana extract (20) and it was shown that these nanoparticles inhibit the growth of E coli. and S aureus. bacteria. polygonum aviculare L. is a member of the family Polygonaceae-Dock. This plant grows in Africa, Europe, Asia, and especially the Middle East. The aforementioned plant species is used to manage diarrhea, homeostasis and wound healing. In addition, it acts as a diuretic and helps eliminate kidney stones (21). It has been confirmed that this plant has high amounts of antioxidants. This research involved the initial use of Polygonum aviculare L. extract for the eco-friendly production of silver nanoparticles. Subsequently, UV-Vis spectroscopy was utilized to verify the creation of these nanoparticles., and Morphological investigation by TEM, FE-SEM, XRD, and FT-IR to examine functional groups in nanoparticles and investigation of antioxidant properties of silver nanoparticles produced by DPPH assay and investigation of their antibacterial properties by Disk Diffusion method.

Materials and Methods

Plant Extract Preparation

First, some plants were collected from the campus of Islamic Azad University, Science and Research Branch of Tehran and were identified by the Herbarium Center of Shahid Beheshti University with the herbarium code HSBU-2021982 for confirmation of botany and then dried in the shadow for 3 days and powdered. Maceration was used to extract this plant. $5g$ of p.avicule L. powder weighed by a sensitive scale and poured into an Autoclaved screw bottle and then 100 ml of mixed solvent (70 ml of Absolute ethanol + 30 ml of Distilled water) was prepared for the hydroalcoholic extract to be added to it and the solution was placed in the water bath with 80-90 $^{\circ}C$ for $45minutes$ and the extract solution was filtered using Watman Filter Paper No. 2. After extraction, the extract was concentrated and the extract solvent was removed by rotary vacuum evaporation (Rv 10 Digital, Germany). and After using the rotary, to dry the extract at 25 $^{\circ}C$(room temperature), it was poured into a watch glass and placed in a suitable place to dry. The extract powder was then poured into a suitable container and kept at a suitable temperature until use.

Synthesis of silver nanoparticles

For the synthesis of silver nanoparticles 10 ml of the extract $90ml$ of silver nitrate ($AgNO_3$) (German company Merck) 2 mM was added and to reduce silver ions, the solution was kept at 25°C and kept in the dark overnight (23). The alteration of the solution's hue from a light-yellow shade to a deep brown or black hue serves as evidence of the creation of silver nanoparticles (24). Subsequently, the solution comprising the nanoparticles underwent centrifugation at 8000 revolutions per minute for a duration of 20 minutes, following which the supernatant was decanted and the remaining sediment was dried. The obtained silver nanoparticles were stored inside the microtube at 4 $^{\circ}C$ for further study (25).

Characterization of AgNPs

Ultraviolet-visible spectroscopy to identify AgNPs

To detect the presence of silver, UV-Vis spectrophotometry (PG instrument two-beam) was utilized to scan the absorption peak within the 350-700 nm range, as silver exhibits absorption within this range.

X-ray diffraction (XRD)

The identification of the crystallography and dimensions of the produced nanoparticles was accomplished through the utilization of XRD analysis in this investigation. Specifically, a copper-based XRD instrument located within the Plasma Physics Complex at the Islamic Azad University, Science and Research Branch of Tehran was employed for the characterization of the synthesized silver nanoparticles.

Infrared Fourier transform Spectroscopy (FTIR)

This technique is used to identify AgNPs functional groups in the range 400-4000 cm $^{-1}$.

Field Emission Scanning Electron Microscope (FESEM)

The morphology of silver nanoparticles synthesized was examined by the Xmu-Mira FESEM. To produce the imaging sample, the gold-covered nanoparticles were utilized to establish surface conductivity, thereby ensuring that the direction of the backscattered electron beams remained unaltered.

Transient electron microscope (TEM)

To determine the size and distribution dispersion of the synthesized silver nanoparticles, the extract of *polygonum aviculare L.*plant, 912AB-LEO model electron microscope with an applied voltage of 120 kV was used to emit an electron beam.

Evaluation of antioxidant properties by free radical scavenging (DPPH)

To evaluate the antioxidant activity of synthesized silver nanoparticles the DPPH solution 0.004% was prepared. Then concentrations of 10, 50, 75, and 100 $mg/L$of nanoparticles were treated with DPPH solution. After being kept in the dark for half an hour, the absorbance of the sample was measured via UV-vis spectroscopy at a wavelength of 517 nm. Ascorbic acid was employed as a standard antioxidant reference purpose. The scavenging activity of free radicals was determined using the following formula: %RSC = $\frac{Ab-As}{\mathrm{Ab}}$ $\times 100$

$\mathrm{A_b}$= DPPH Absorbance, $\mathrm{A_s}$= Sample or Standard Absorbance

The antioxidant activity of each sample was expressed in terms of $\mathrm{IC_{50}}$ (concentration required to inhibit the 50% DPPH radical formation) calculated from the inhibition curve.

Antibacterial assay

The agar disk diffusion method was utilized to assess the antibacterial effectiveness of the silver nanoparticles that were produced. This was done to confirm the antibacterial properties of the synthesized silver nanoparticles. Nutrient agar medium was made and sterilized, and 10 ml of culture medium was added to each Petri dish. After freezing the culture medium, the investigated bacteria were cultured in nutrient agar culture medium on Petri plates. Then, different concentrations of synthesized silver nanoparticles (5, 10, 20, 40 (%v/v)) were poured on 6 mm discs (Pad tan Teb) sterilized in an autoclave and on the nutrient agar plates utilized and then incubated at 37°C for a period of 24 hours. Following this, the diameter of the growth inhibition zone is measured in millimeters (39,41).

Data Analysis

Antioxidant evaluation and antibacterial were repeated 3 *times*and $\mathrm{IC_{50}}$ values were inhibited by linear regression between percent and Relevant concentrations were obtained. Graph Pad Prism 9, Excel 2013 software, and the ANOVA one-way test was employed to examine the findings, and the level of significance was set at $p < 0.05$.

Results

According to Figure 1, the initial sign of silver nanoparticle synthesis is the changeable of the sample color from light yellow to dark brown.

Figure 2 showed the results of UV-Vis spectroscopy. According to reference (26), the presence of silver nanoparticles can be inferred from the absorption peak occurring at a wavelength of 430 nm. This is because silver tends to absorb light within the range of 400-450 nm.

The X-ray diffraction (XRD) pattern displayed in Figure 3 corresponds to the synthesis of silver nanoparticles using the hydroalcoholic extract of the plant. The pattern shows peaks at 2ϑ values of 23.35°, 27.56°, 32.04°, and 46.00°, which are indicative of the spherical shape of the nanoparticles. Moreover, the XRD pattern matches perfectly with the reference pattern (JCPDS No. 04-0783) for silver nanoparticles. The formula provided can be used to determine the crystallite size of silver nanoparticles:

$$D = \frac{\mathrm{k}}{\mathrm{cos}}$$

In the above relation, K = 0.9 was the form factor, λ The X-ray wavelength is equal to 1.5406 *angstroms* and β is the full width at half the maximum peak diffraction, ϑ is the angle corresponding to the diffraction. From this calculation, the size of silver nanoparticles was approximately 29 nm (28.46nm).

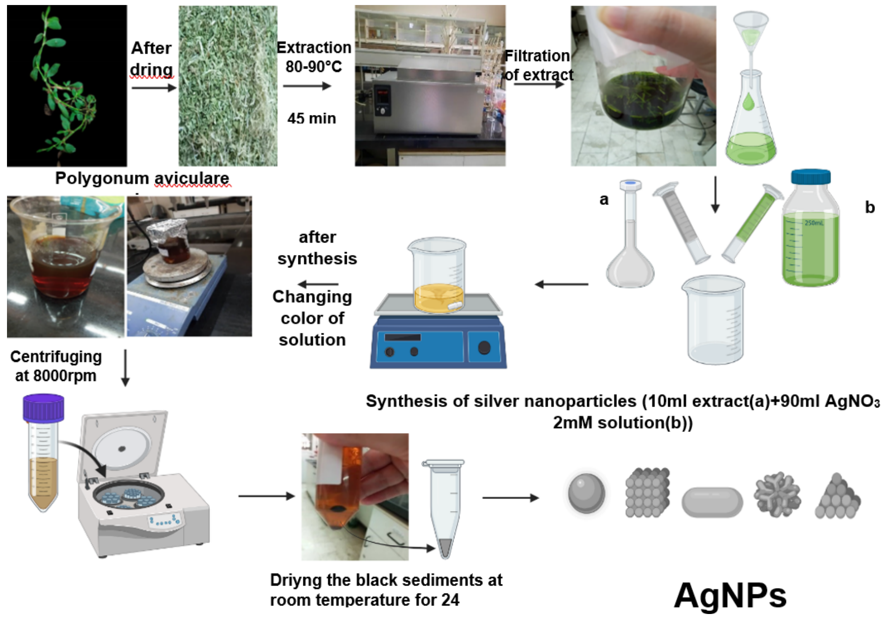
To determine the functional groups responsible for the synthesis of silver nanoparticles in Polygonum aviculare extract, its hydroalcoholic extract was analyzed using FT-IR spectroscopy. Figure 4 presents the FT-IR spectrum of this plant extract, where the broad and intense peak observed at 3416.09 cm-1 represents the stretching vibration of the hydroxyl group (OH) present in water, phenolic compounds, and flavonoids. The peak observed at 2926.40 cm-1 corresponds to the presence of alkyl and CH groups, whereas the peak at 1616.91 cm-1 is associated with the C=O bond of the amide group and the C=C bond of aromatic rings. Additionally, the peaks at 1060.81 cm-1 are related to the presence of ether connections. The FT-IR analysis also revealed the presence of various functional groups, such as hydroxyl, carboxyl, and carbonyl, on the surface of the nanoparticles, which contributed to their negative charge (as shown in Figure 4). The peak at 1604 cm-1 is attributed to the C=C stretching vibration of aromatic rings, and the peak at 1360 cm-1 corresponds to C–H bending. The peaks observed at 1061 cm-1 are related to the C-O tensile vibration bonds in the ester and acid groups, and the bonds at 2839/13 and 2921/88 cm-1 correspond to the C-H tensile bond in alkane compounds. The strong peaks at 3400 cm-1 correspond to -OH tensile due to the presence of phenolic compounds in the Polygonum aviculare L. extract. The intensity of most bands is reduced due to the reduction of silver ions during the synthesis of silver nanoparticles.

Figure 5 showed the FESEM image of the nanometer dimensions of silver particles and shows the spherical shape at all magnifications. According to FESEM images, the cumulative size of nanoparticles varies between 40 and 70 nm .

Figure 6 showed the transmitting electron microscope TEM of silver nanoparticles synthesized with *polygonum aviculare L.* extract. As can be seen from the pictures, silver nanoparticles were in a darker image and have a spherical shape and a good distribution and around the nanoparticles, a light background is observed, which is related to the solvent, and because the density of the solvent against the passage of light is lower than the density of silver nanoparticles, and therefore silver nanoparticles are darker and solvent lighter in the image.

Figure 7 and Table 1 showed the Results of the antioxidant properties of silver nanoparticles. The synthesized showed that the 50$\%$ free radical scavenging (IC_{50}) inhibition rate of the synthesized nanoparticles was calculated to be 15.63$mg\ /L$ Compared to the standard antioxidant ascorbic acid with IC_{50} equal to 11.89 mg /L showed excellent antioxidant activity.

Figure 8 showed that synthesized silver nanoparticles by Polygonum aviculare L. extract had more antibacterial properties against Gram-negative E coli. than Gram-positive S. aureus, the highest growth inhibitory zone corresponds to the concentration of 40 (%v/v) of synthesized silver nanoparticles and the lowest growth halo corresponds to the concentration of 10 (%v/v) (Table 2).



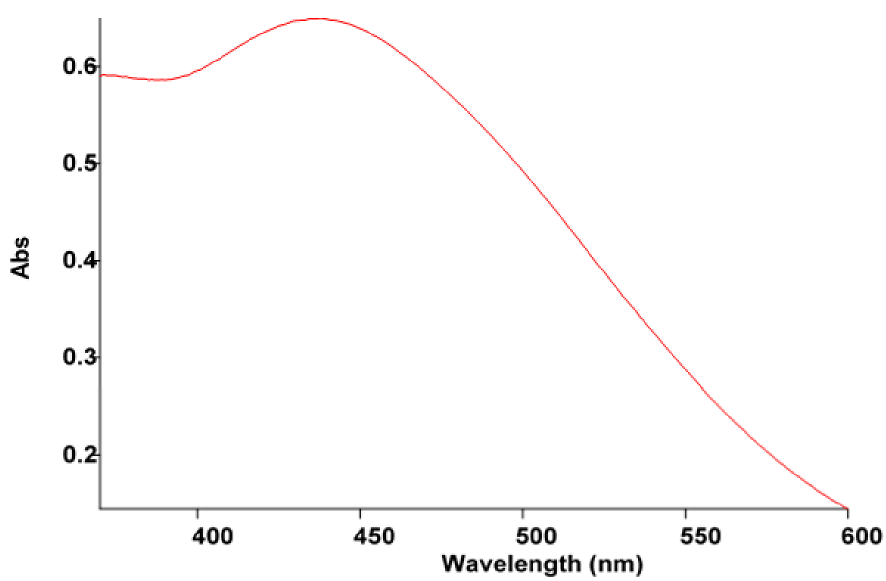


Figure 1. illustrates the process of synthesizing AgNPs from p. aviculare L. by green method.

Figure 2. The UV-visible spectrum of the synthesized silver nanoparticles displayed an absorption peak at 430 nm, signifying the successful formation of the nanoparticles.

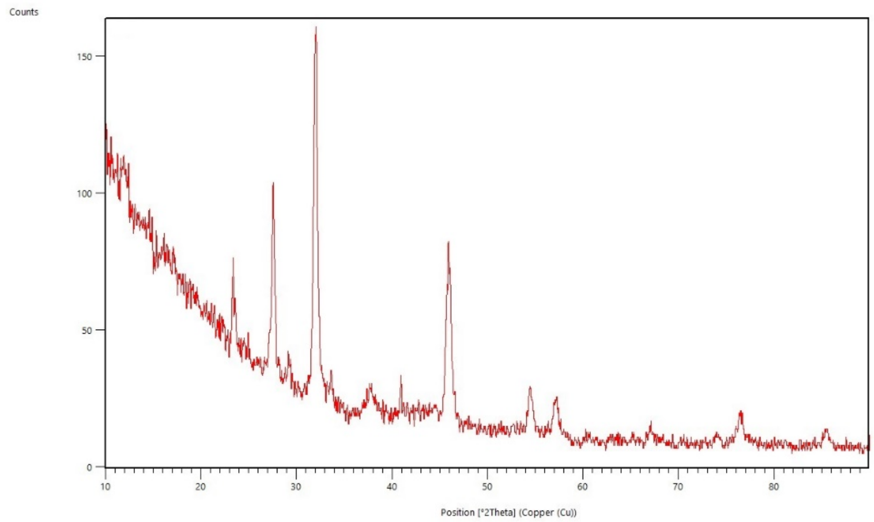


Figure 3. Analysis of the X-ray diffraction spectrum (XRD): It has strong and clear peaks that indicate the crystallization and high purity of the synthesized zinc oxide nanoparticles.

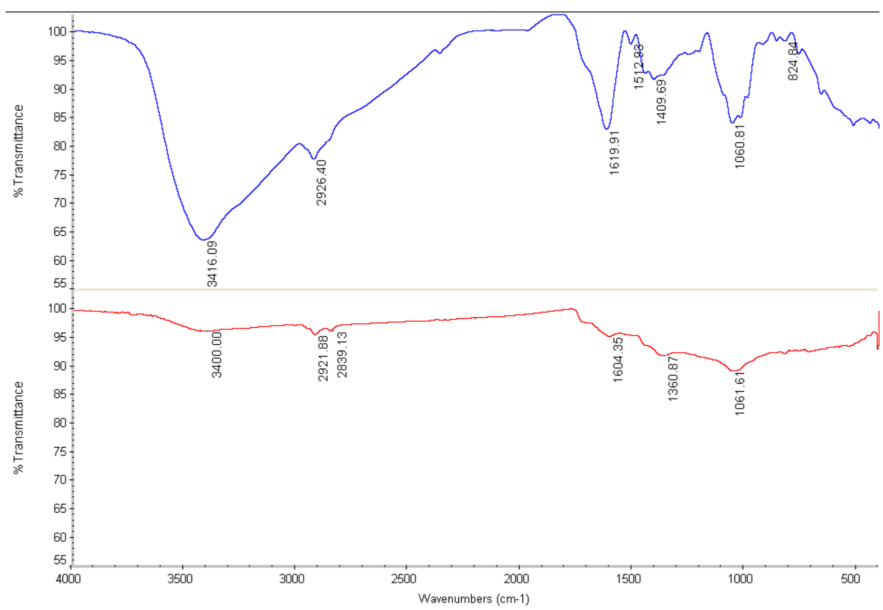
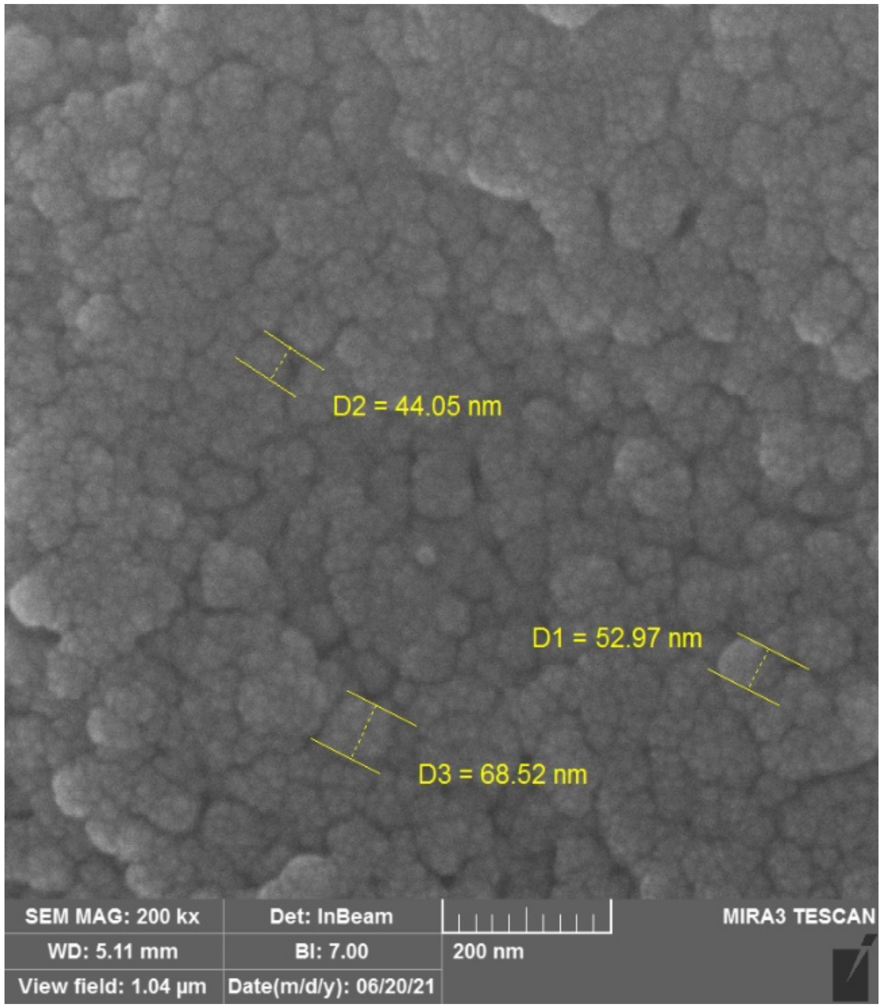
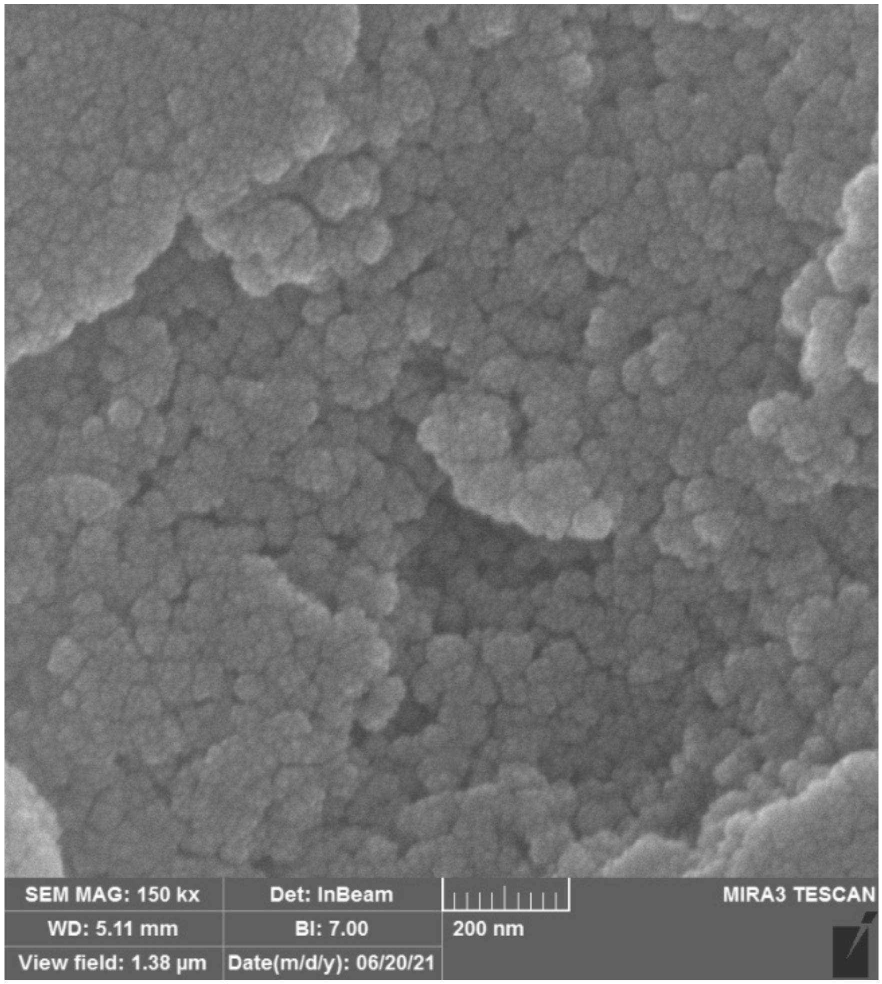
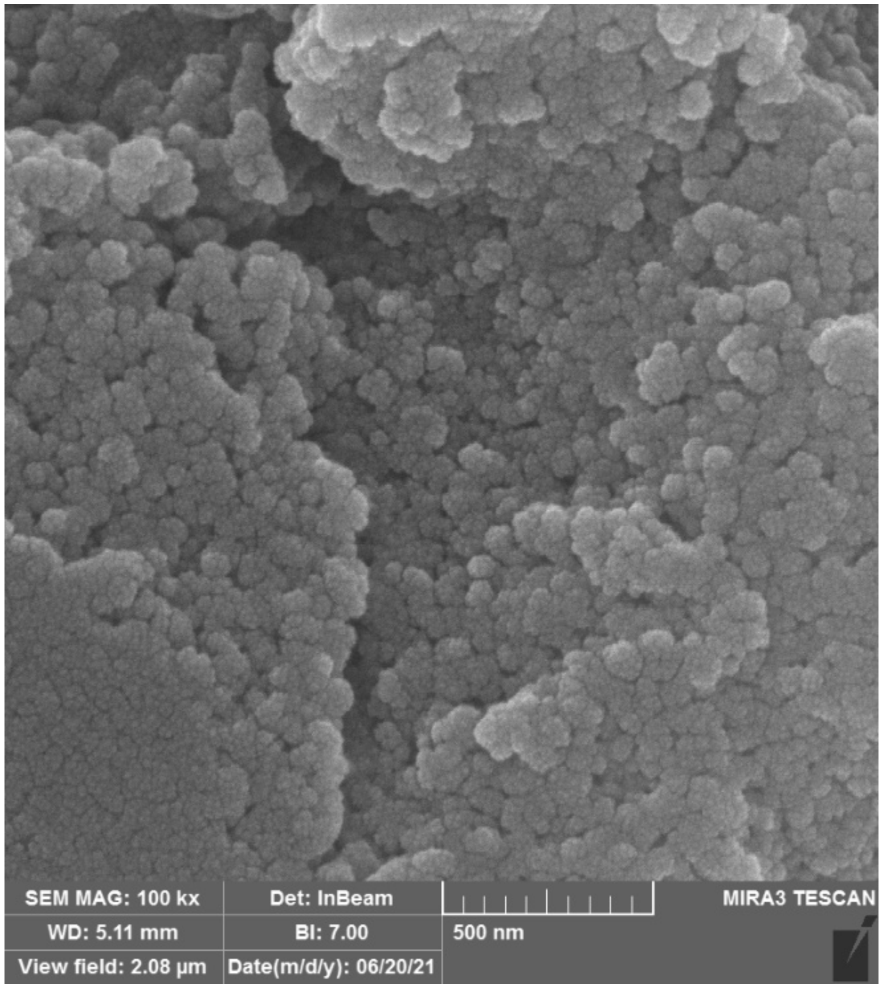


Figure 4. FT-IR spectrum of (a) hydroalcoholic P. aviculare L. extract and (b) synthesized Ag nanoparticles. As depicted, the intensity of most bands is decreased due to a decline in Ag ions.


D2 = 44.05 nm
D1 = 52.97 nm
D3 = 68.52 nm
SEM MAG: 200 kx
Det: InBeam
MIRA3 TESCAN
WD: 5.11 mm
BI: 7.00
200 nm
View field: 1.04 μm
Date(m/d/y): 06/20/21


SEM MAG: 150 kx
Det: InBeam
MIRA3 TESCAN
WD: 5.11 mm
BI: 7.00
200 nm
View field: 1.38 μm
Date(m/d/y): 06/20/21


SEM MAG: 100 kx
Det: InBeam
MIRA3 TESCAN
WD: 5.11 mm
BI: 7.00
500 nm
View field: 2.08 μm
Date(m/d/y): 06/20/21

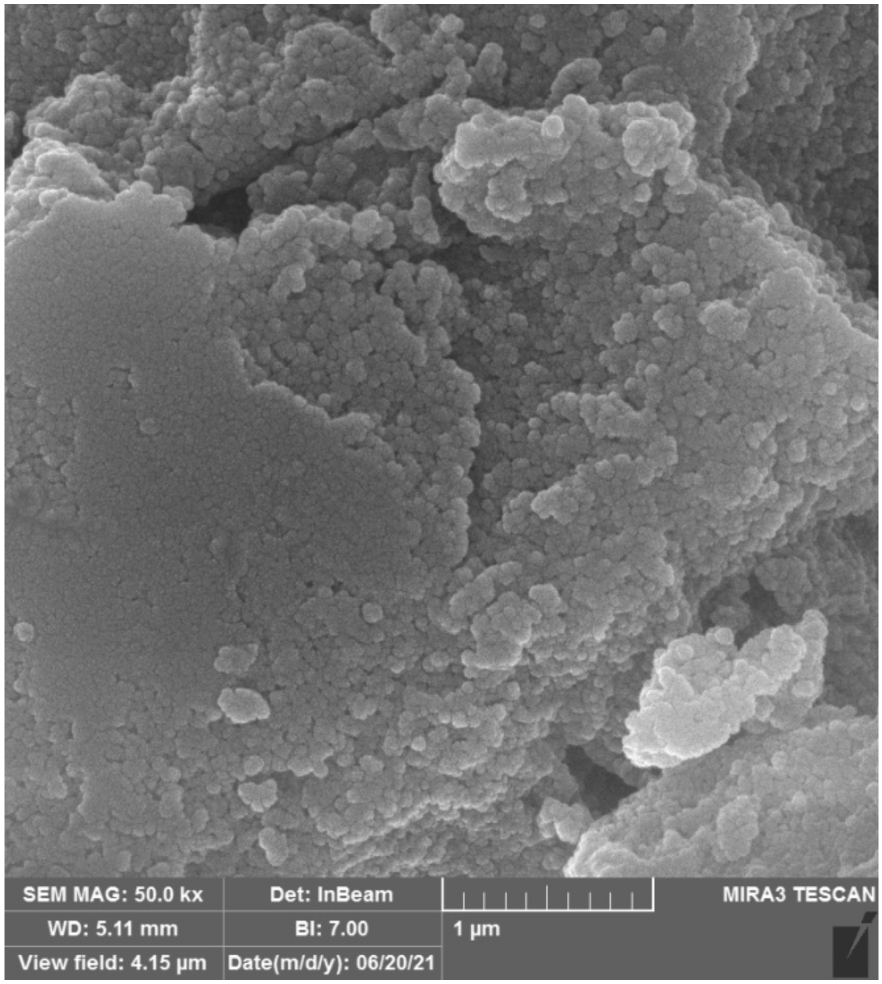


Figure 5. FESEM image of Silver Nanoparticles Synthesized with*polygonum aviculare L.* extract with different magnifications. Ag nanoparticles were mostly spherical., and the size was found to be between 44 and 69 *nm* .

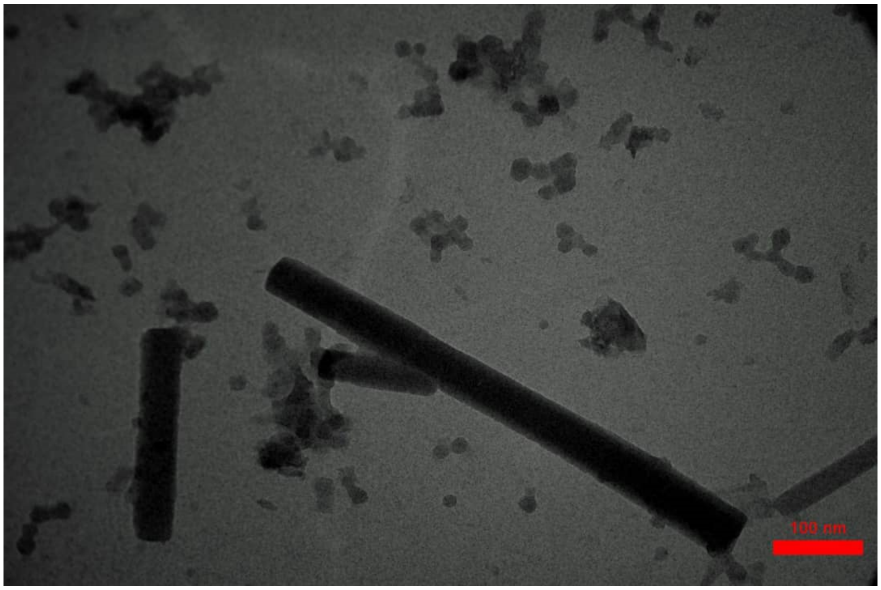


Figure 6. TEM image of Silver Nanoparticles Synthesized with*polygonum aviculare L.* extract. Ag nanoparticles were spherical and rod shaped.

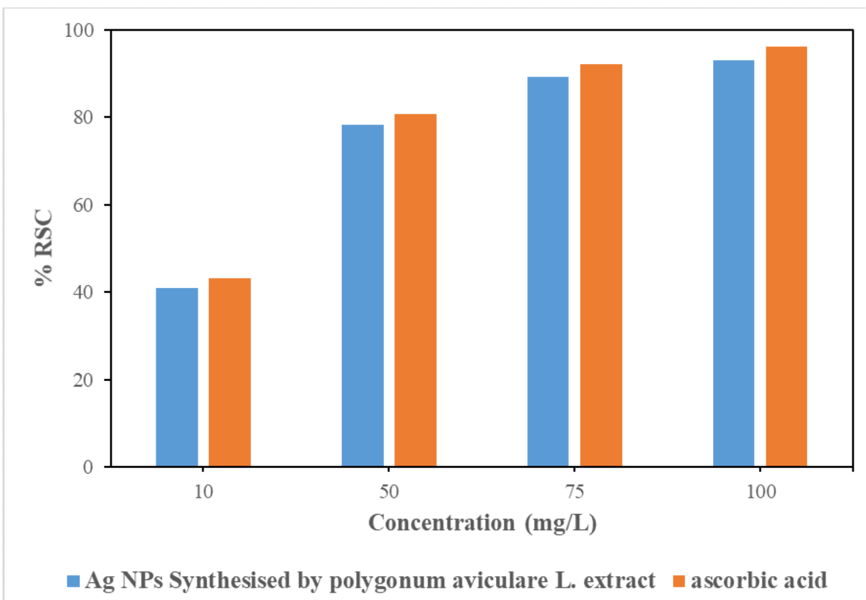


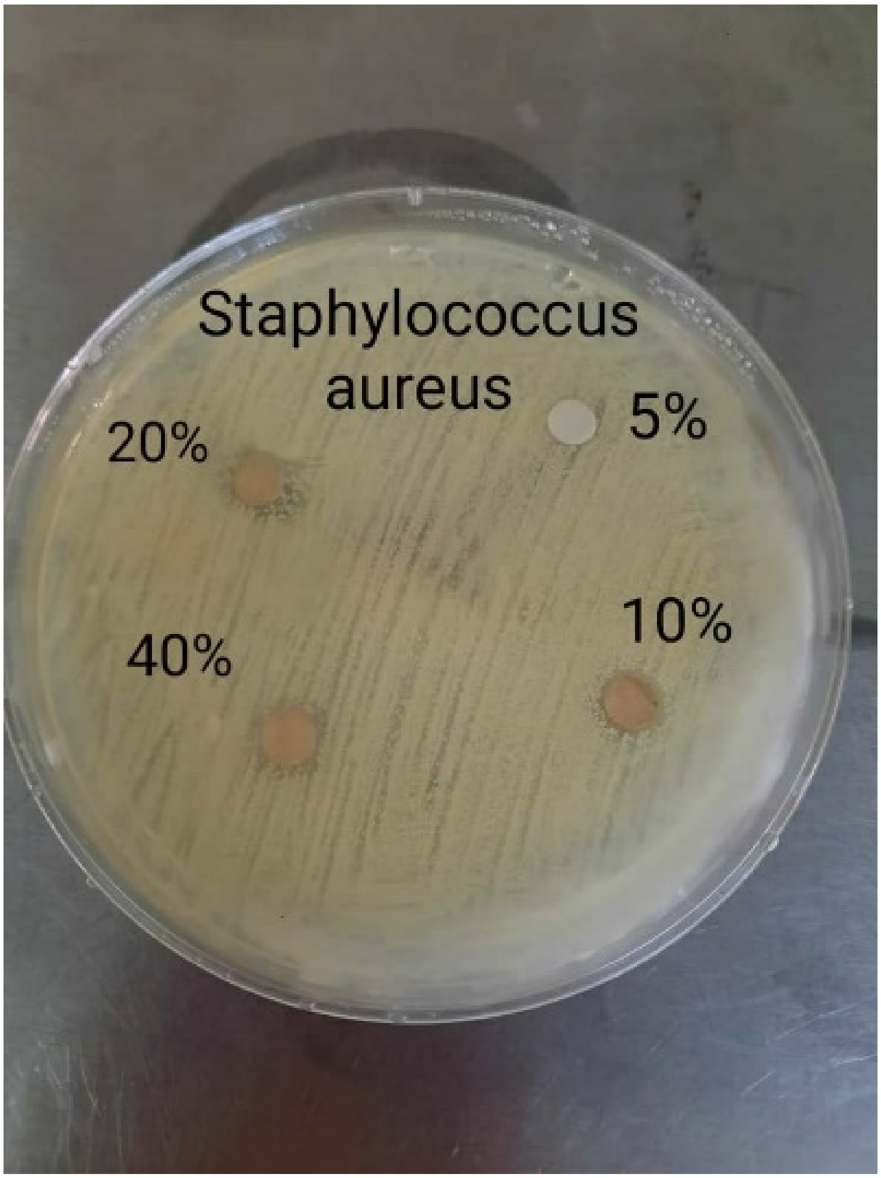
Figure 7. The free radicals scavenging activity of DPPH under the influence of nanoparticles synthesized by hydroalcoholic extract of *polygonum aviculare L.* in comparison with positive control of standard ascorbic acid in different concentrations. The comparison between AgNPs and ascorbic acid demonstrated a noteworthy difference, with a statistical significance of $P < 0.005$.

Table 1. Percent of free radical scavenging by different concentrations of nanoparticles synthesized by hydroalcoholic extract of

polygonum aviculare L.

Concentration of extract (mg / L)	Absorbance	%Percent of inhibition of extract
10	0.3952	41.01%
50	0.1452	78.32%
75	0.0714	89.34%
100	0.0460	93.13%

As seen, with the increased concentration of the synthesized silver nanoparticles, their inhibition rate has also increased.



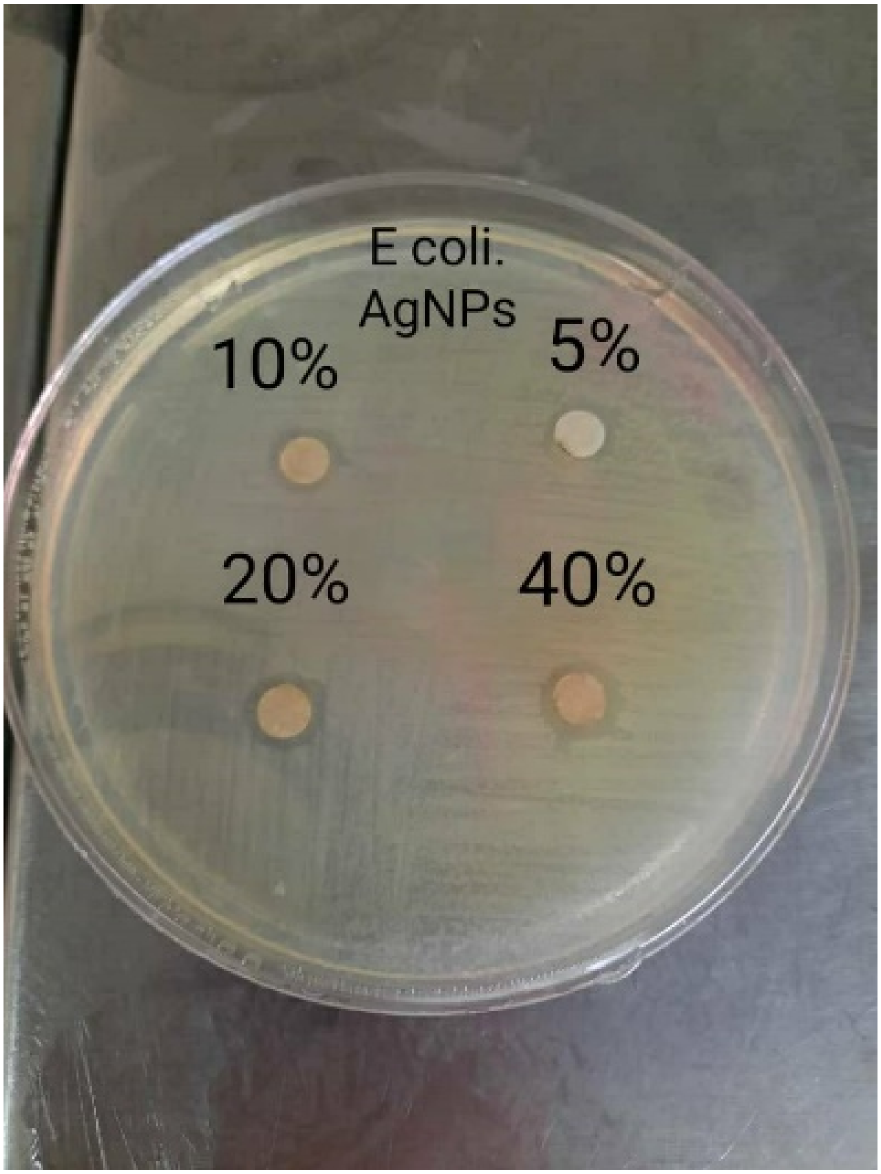


Figure 8. The inhibition area of AgNPs against E. coli and S. aureus indicated that the antibacterial activity of AgNPs was more effective against E. coli compared to S. aureus.

Table 2. Results of mean diameter (mm) of non-growth halo of Nanoparticles Synthesized with hydroalcoholic extract of *polygonum aviculare L.* on bacteria

Concentration (% V/V) / Bacteria strains	5%	10%	20%	40%
E Coli.	8 mm	9 mm	10mm	11mm
S. aureus	0	7 mm	9 mm	10 mm

The highest rate of non-growth of bacteria is related to Escherichia coli bacteria with a halo diameter of

11 mm at a concentration of 40 ($\%\ V/V$) and the lowest rate of non-growth of bacteria is related to Staphylococcus bacteria with a halo diameter of 7 mm at a concentration of 10 ($\%V/V$).

Discussion and conclusion

The synthesis of nanomaterials is at present one of the most active fields in the field of nanosciences. One of the nanomaterials is silver nanoparticles, which have known inhibitory and antibacterial effects. Silver ions can bind to electron donor groups such as glucose, oxygen, or nitrogen in a biological molecule (22). Silver nanoparticles break down the inhibitory components in the outer membrane of the bacterium, releasing molecularly exponential molecules such as the liposuction molecule. After penetrating the bacterial cell, nanosilver inactivates its enzymes and causes the death of the bacterium by producing hydrogen peroxide (23). Various techniques exist for synthesizing nanoparticles, but physical and chemical methods can cause environmental pollution. To mitigate this issue, green methods can be employed, where materials with minimal environmental impact are either produced or consumed as an alternative (24). Furthermore, research has demonstrated that the presence of silver ions can significantly enhance the generation of reactive oxygen species, such as superoxide anion radicals, which can induce oxidative stress at the molecular, cellular, and organ levels (25). Plants have antimicrobial and antioxidant activity due to the presence of secondary metabolites such as phenol and flavonoids which act to prevent oxidative damage to cells. Certain plants can convert Ag^{+} ions to Ag^{0}, synthesizing nanoparticles with antioxidant properties through a process called bioremediation (26). It has antimicrobial and antioxidant activity and acts to prevent oxidative damage to cells. Researchers have increasingly viewed the synthesis of nanoparticles by plants and microorganisms as a biocompatible and environmentally friendly approach in recent years. The study examined the abilities of the nanoparticles that were created to act as antioxidants and antibacterials. During the study, a change in color was observed from light yellow to dark brown, which was caused by the interaction between the plant extract and silver salt solution. This color change was consistent with the findings of Rezazadeh et al. (27), Givianrad et al. (28), and Dousti et al. (29), and it served as the initial indication of the production of silver nanoparticles. Dousti et al. conducted Uv-Vis spectroscopy on the silver nanoparticles they synthesized and found that the maximum absorption occurred around 430 nm. The presence of a peak in this region is an indication of silver nanoparticle synthesis because such a peak, occurs at a wavelength of 400 - 450 nm, this phenomenon is caused by the induction of free electrons within the nanoparticles. The surface plasmon resonance observed in silver nanoparticles is linked to the induction of unbound electrons within the nanoparticles. This effect is responsible for the phenomenon. This outcome aligns with the discoveries made by Dousti et al. and other researchers (29). Also, Dousti et al. synthesized silver nanoparticles from the blue extract of the Fumaria Parviflora plant, and their XRD analysis showed peaks at 38.07°, 44.26°, 64.43°, and 77.35° with indices (311), (220), (200), and (111), respectively. These peaks correspond to the nanostructures of the silver particles and are in complete agreement with the XRD pattern of silver. The size of the silver nanoparticles' crystallinity was determined using Debye Scherrer's formula, which yielded a size of 30 nm for the nanoparticles. as well as the X-ray diffraction (XRD) pattern obtained from the nanoparticles created using Polygonum aviculare L. indicated that the characteristic peaks at 2ϑ values of 23.35°, 27.56°, 32.04°, and 46.00°, which are attributed to the spherical shape of silver nanoparticles and agreed with the XRD reference pattern of silver nanoparticles (JCPDS No. 04-0783). as well as, the size of the silver was determined to be 29nm using Debye-Scherrer's equation, which agreed with the findings of Dousti et al.'s study (23, 30, 8, 29). Heydarzadeh and colleagues conducted a study on the eco-friendly production of silver nanoparticles using Citrus aurantium extract. The transmission electron microscope (TEM) images revealed that the silver nanoparticles had a spherical shape and appeared darker in the images. The nanoparticles obtained had a diameter ranging from 5 to 40 nm. FESEM images of silver nanoparticles synthesized with orange spring extract with different magnifications also showed the nanometer dimensions of the silver particles and indicated an almost spherical shape in all magnifications. According to the FESEM images, the cumulative size of the nanoparticles was between 10 - 60 nm . also, in this study, the FESEM image of the nanometer dimensions of silver particles shows the spherical shape at all magnifications. According to FESEM images, the cumulative size of nanoparticles varies between 40 and 70 nm , and the TEM image of silver nanoparticles synthesized from polygonum aviculare L. extract showed the silver nanoparticles were in a darker image and had a spherical shape and a size of less than 50 *nm* which It was in good agreement with the results of Heydarzadeh and his colleagues (40). Since it is important to find both natural and synthetic antioxidants to prevent oxidative stress and its harmful effects, the study also aimed to examine the antioxidant properties of the produced nanoparticles. The study showed that the synthesized nanoparticles were able to remove radicals the free 2 and 2-diphenyl-1-picryl is hydrazyl in such a way that by receiving an electron or free radical, hydrogen is converted into a stable molecule. Polygonum aviculare plant can transfer hydrogen to oxidants and antioxidant properties so the results showed that the antioxidant properties of synthesized nanoparticles depend on their concentration and with increasing concentration, antioxidant activity increases. The silver nanoparticles exhibited remarkable antioxidant activity, with an IC_{50} value of 15.63 *mg/l* , when compared to the standard antioxidant ascorbic acid, which had an IC_{50} value of 11.89 *mg/l* . This discovery aligns with the outcomes observed in earlier investigations (31). Abdolaziz and colleagues (32) conducted a study on the antioxidant activity of silver nanoparticles synthesized from the leaf extract of the Salmak plant using the DPPH test. The results revealed that the antioxidant effect was directly proportional to the concentration of silver nanoparticles, with an increase in concentration up to 20 mg/l resulting in a measurement of 63.45 $\pm$ 0.18 *mg/l* . Our research findings indicated that the inhibitory ability of nanoparticles produced using the hydroalcoholic extract of Polygonum aviculare L. plant was 41.01 at a concentration of 10 mg/l, and it increased to 93.13 at concentrations up to 100 mg/l, with statistical significance at $p < 0.05$. This result is in line with previous studies (33, 34) and demonstrates a notable variation. When used at a concentration of 40% (%v/v) against S. aureus and E. coli, the average diameter of the growth inhibition zone was 10 mm and 12 mm, respectively. The research established that silver nanoparticles produced via the green method were more highly efficient in inhibiting the growth of gram-negative bacteria, like E. coli than gram-positive bacteria, such as S. aureus. This observation is consistent with the findings of Mobarak Ali et al. (36) and (35), who suggest that the thicker cell wall of gram-positive bacteria, such as S. aureus is the reason for this result. In contrast, gram-negative bacteria have a thinner cell wall, and their outer surface is covered by a layer of lipopolysaccharide. Due to the weak positive charge of silver nanoparticles, their interaction with bacteria is facilitated by the negative charge present on the surface of these microorganisms. This interaction is thought to result in the creation of a hole in the cell wall, which can ultimately lead to the entry of nanoparticles into the bacterial cell and the bacterium's subsequent death. Also Vastava et al. (37) and Guzman et al. (38) reported in their milk studies that the antibacterial effectiveness of nanoparticles increases as their size decreases. Therefore, the difference in the minimum inhibitory concentration and the minimum bactericidal concentration in the synthesized nanoparticles Can also be attributed to the difference in size. This difference in the results can also be related to the type of nanoparticle shape as well as the structural and genetic differences of the strains according to the geographical location. Silver nanoparticles from common drugs can be a suitable alternative for them. The method utilized in this investigation to synthesize silver nanoparticles from the P. aviculare L. extract is regarded as an advantageous approach eco-friendly. Cost-effectiveness, avoidance of toxic solvents, waste and antimicrobial activity, and other biomedical applications allow this method to be used in large commercial comparisons.

Acknowledgments

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