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Research Article

Green synthesis of silver nanoparticles using a combination of *Allium sativum* and *Peganum harmala* aqueous extracts and evaluating its antibacterial activity

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ABSTRACT

The resistance of bacteria to antibiotics has significantly increased due to their widespread and improper use. The emergence of multidrug-resistant (MDR) strains has made the treatment of infections more challenging, resulting in higher healthcare costs and mortality rates. This issue underscores the urgent need to develop alternative therapeutic approaches. This research was carried out in order to biosynthesis and identify silver nanoparticles using the combination of *Allium sativum* and *Peganum harmala* plant extracts and to evaluate their antibacterial effect.The characterization of the biosynthesized silver nanoparticles was performed using methods dynamic light scattering (DLS), visible-ultraviolet spectroscopy (UV-Vis), scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and Energy-Dispersive X-ray (EDAX). The antimicrobial function of biosynthesized AgNP was also investigated using microdilution method against pathogenic Gram-positive Bacteria *Staphylococcus aureus* and Gram-negative bacteria, *Escherichia coli*, *Salmonella Typhimurium*, *Pseudomonas aeruginosa*. The results were finally analyzed using SPSS software. The SEM image shows spherical nanoparticles with a size of 40 to 45 nanometers. The biosynthesized nanoparticles show significant antibacterial effect, so that their minimum inhibitory concentration on *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella Typhimurium* and *Escherichia coli* were reported to be 62.5, 15.7, 31.25 and 62.5 ppm respectively ($p \leq 0 / 05$). According to the obtained results, silver nanoparticles prepared using mixed plant extracts of *Allium sativum* and *Peganum harmala* have a significant antimicrobial effect on Gram- negative and positive bacteria therefore can act as natural antimicrobial compounds.

1. Introduction

Bacteria are important common cause of chronic infections and mortality. *Staphylococcus aureus* is a major human pathogen responsible for a wide range of infections, including skin and soft tissue infections, pneumonia, and sepsis. *Escherichia coli* is a leading cause of gastrointestinal diseases and urinary tract infections. *Salmonella Typhimurium* is a significant causative agent of foodborne illness, leading to gastroenteritis (Mandal et al., 2010).

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Pseudomonas aeruginosa is an opportunistic pathogen that primarily affects immunocompromised individuals, causing chronic infections in respiratory and urinary systems, and is known for its resistance to many antibiotics (Oussalah et al., 2007). To deal with them, antibiotics are used as a desirable treatment method due to their affordability and effective results. However, Studies show that in recent years, inappropriate use of antibiotics has caused the emergence of multi-drug resistant bacterial strains (Salam et al., 2023). The main issue worrying global public health issues at present is the antibiotic resistance crisis (Hsueh, 2010). Therefore, new research and investigation should be done all over the world to produce more effective antimicrobial compounds.
Among the factors that have been considered as an alternative to antibiotics in recent years are metal nanoparticles that have a significant antimicrobial effect(Ghaderi et al., 2021; Wahab et al., 2023). Nano has become one of the most important and effective technologies on science in the world. Nanomaterials, defined as particles ranging in size from 1 to 100 nanometers, have garnered significant attention due to their unique properties and wide-ranging applications (Mekuye & Abera, 2023). The antibacterial effects Nanoparticles are created due to the presence of specific physicochemical properties in nanoparticles (Wasilewska et al., 2023). Including more interaction with cells due to the developed surface, which provides maximum contact with the environment and the ability to control their size, stability and shape (More et al., 2023).

silver nanoparticles have many important applications. In recent years, it is used as an antimicrobial agent (Xu et al., 2020). According to past studies, silver ion is an impressive antimicrobial factor and has a significant antimicrobial effect ON pathogenic factors such as bacteria (Mohamed et al., 2020). The synthesis methods of AgNPs are biological, chemical and physical. Chemical and physical technique are usually not used in medicine due to the production of toxic products that are hazardous for the environment Because they may create health issues, exclusively in the clinical field (Iravani et al., 2014).. In recent years, the synthesis of environmentally friendly nanoparticles, which do not produce toxic products, has been developed (Dhaka et al., 2023). Green synthesis means synthesizing nanoparticles through biological pathways such as yeast, plants, bacteria and fungi, their by-products as using various biotechnological techniques (Hano & Abbasi, 2021). This eco-friendly process not only minimizes environmental risks but also ensures biocompatibility, making it ideal for applications in medicine, agriculture, and environmental remediation (Ahmed et al., 2016). As a sustainable alternative, green nanoparticle production addresses the growing demand for safer and more responsible nanotechnology solutions (Gour & Jain, 2019). Recently, the utilize of plant extracts has much consideration because of its simplicity, lowest level cost , high efficiency,non-toxicity, and compatibility with the environment compared to other methods (Osman et al., 2024). Plants are a nice alternative for Green synthesis of AgNPs due to their abundance and availability (Kulkarni et al., 2023).

Garlic *(Allium sativum)* is a perennial plant in the family Amaryllidaceae contains a variety of minerals, vitamins, flavonoids, volatile and non-volatile compounds and is therapeutically used by humans as far (Robles Martínez et al., 2019). *Allium sativum* is a widely distributed plant cultivated globally for its culinary and medicinal properties. Originating from Central Asia, it has adapted to diverse climates and is now a major crop in countries like China, India, and the United States. In Iran, garlic is primarily grown in regions such as Hamedan, Mazandaran, and Khorasan, known for their fertile soil and favorable conditions. The plant's adaptability and significance in traditional medicine have made it a valuable crop worldwide (Block, 2010). Another medicinal plant *P. harmala* belongs to the family Zygophyllaceae and has many applications as a therapeutic agent. Seeds of the plant contain 2 to 6% active pharmaceutical alkaloids, most of which are beta-carboline compounds including harmane, harmine, harmaline and harmalol (Davarnia et al., 2020). The key research question is*:* Can silver nanoparticles synthesized using a combination of Allium sativum and Peganum harmala extracts serve as effective antimicrobial agents against pathogenic microorganisms? This work seeks to provide a sustainable alternative for AgNP synthesis while

exploring the synergistic effects of these plant extracts in combating microbial resistance.

2. Materials and Methods

Allium sativum and *Peganum harmala* were collected in spring around Lorestan province he plants was collected during the mature stage, which is typically when reaches its full growth potential. High purity silver nitrate salt was obtained from Merck (Germany) and standard bacterial strains of *Escherichia coli* (PTCC 25923), *Salmonella typhimurium (Available in mycology laboratory of Razi University)*, *Pseudomonas aeruginosa* (ATCC (R613)) and *Staphylococcus aureus* (PTCC 25922) were obtained from Iran Science and Technology Organization (IROST). Each experiment was performed in triplicate.

2.1 Preparation of the extract

Fresh leaves and roots of *P. Harmala* and *A. sativum* were collected, washed and dried at 25°C and powdered. 5 grams of the powder of each plant was weighed and poured into 100 ml of water while boiling. Mix well and boil for 10 minutes, then the extract was centrifuged at 5000 rpm for ten minutes The prepared plant extracts were purified twice with Whatman filter paper(Vanlalveni et al., 2021).

2.2 Green Synthesis of silver nanoparticles

In the beginning silver nitrate solution (1 mM) was prepared. 10 ml of mixed extract including (7 ml of *Allium sativum* and 3 ml of *Harmala Peganum* extract 2000ppm) was mixed with 90 milliliters of 1 mill molar AgNO3 solution. Then mix was exposed to sunlight for 10 minutes to obtain a color change. The color change to light brown confirms the reduction of Ag to Ag NPs. Color change is the first sign that confirms the biosynthesis of NPs. After the reaction, the remaining solution was 10 min centrifuged (rpm) at 12000 until remove unreacted substances(Velmurugan et al., 2024).

2.3 Characterization of Silver Nanoparticles

The characteristics of the synthesized silver nanoparticles were obtained using spectrophotometric uv-vis, DLS, SEM, EDX, FTIR, and XRD tests (Forough & Farhadi, 2010).

2.4 Antibacterial assay

The antibacterial activity of different concentrations of 500, 250, 125, 62, 31, 15 and 7 ppm of synthesized silver nanoparticles was investigated using disc diffusion and broth microdilution methods on the pathogenic bacteria *Staphylococcus aureus*, *Escherichia coli*, *Salmonella Typhimurium*, and *Pseudomonas aeruginosa*.

2.4.1. Disk diffusion test

the agar diffusion test was utilizing to measure the sensitivity of bacteria to antibiotics, plant extract and biosynthesized nanoparticles. In this test, small discs impregnated with antibiotics, nanoparticles and extracts were used. This test involves placing paper disks saturated with Antibacterial factors on the surface of agar medium containing bacteria, incubating the plate 24 hours and measure the presence or absence of an inhibition zone around the disks. Mueller Hinton (MH) agar medium was used to cultivate bacteria. 50 µ/l of fresh bacterial culture was inoculated into Muller-Hinton agar medium. Sterile paper discs with a diameter of 5 mm (containing different silver nanoparticles concentrations) along with the extract (2 mg/ml) and the standard antibiotic cephalexin (30 µg/ml) were placed in each plate. After 24 hours of incubation at 37°C, the zone of bacterial growth inhibition around the disk was measured (Jorgensen & Turnidge, 2015).

2.4.2. Minimum inhibitory concentration (MIC)

Broth microdilution method was used to determine the MIC of silver nanoparticles. For each bacterium, 4 rows of microplates were assigned and 100 Microliter of Mueller Hinton Broth was transferred to each well. 100 Microliter of AgNPs (500 ppm) was added to the first well of each row and Serial concentrations were obtained. 10 Microliter of McFarland half standard of each bacterium was added to each well. Well 8 was considered as a positive control (containing culture medium + bacterium sample) and row 4 as a negative control (containing different concentrations of

silver nanoparticles). Then the optical absorption of all the wells was taken with ELISA 630 nm, it was for 24 hours incubated at 37°C. After this time, secondary absorption was taken and finally primary and secondary absorption were compared. A well with almost the same primary and secondary optical absorbance was determined as the MIC (Kowalska-Krochmal & Dudek-Wicher, 2021).

Minimum Bactericidal Concentration (MBC)

At first, concentrations (500, 250, 125, 62, 31, 15 and 7 ppm) of silver nanoparticles were prepared and investigated. All experiments were repeated three times and averaged. A well whose primary and secondary absorbance was almost the same means that bacterial growth was prevented and was determined as the MIC. Then two wells before and after the MIC well were cultured and the lowest concentration that actually killed 99.9% of the microorganism was considered as MBC (Kowalska-Krochmal & Dudek-Wicher, 2021).

2.5. Data analysis

SPSS version 16 software was used for data analysis. One-way analysis of variance was used to analyze the data. Then the sig value of the variance analysis table output is checked and if its value is ($p \leq 0.05$), the assumption of equality of means is rejected and there is at least one significant difference between the studied groups. In order to determine which group's mean is different, Scheffe's test and post hoc multiple comparison were used.

3. Results

3.1. Green Synthesis of silver nanoparticles

In this research, silver nanoparticles were biosynthesized using the composition of two plant extracts, *Allium sativum* and *Peganum harmala*. The first indication of biosynthesis of silver nanoparticles is the color change of the solution. The reduce of Ag+ to AgNPs confirmed by changing in the color of the solution. The dark brown color obtained after 15 minutes indicates the biosynthesis of AgNPs in the solution. The color is due to the plasmon surface resonance in metallic nanoparticles (Fig.1).

3.2. Characterization of Silver Nanoparticles

The color change is caused by the excitation of surface plasmon vibrations. The biosynthesized AgNPs in this study at 450 nm had an absorption peak (Fig. 2). The presence of only one sharp adsorption peak in the diagram indicates the mono dispersion and uniform distribution of biosynthesized nanoparticles.

X-ray technique was used to analyze the nature and arrangement of atoms inside crystals of the biosynthesized AgNPs. The XRD technique conducted on the nanoparticles revealed intense peaks corresponding to the crystal planes (2 0 0), (2 2 0), (1 1 1), and (3 1 1) in accordance with Bragg's law, based on the face-centered cubic structure of the silver nanoparticles. The broaden of the Bragg's peaks is indicative of the presence of AgNP (Fig. 3). The crystallite size is calculated using the Debye-Scherer formula $D = (K \times \lambda)/ (\beta \times \cos \theta)$. XRD size represents the size of the crystal grains in the particle and is usually smaller than the size observed in SEM, because SEM shows the entire particle (including multiple crystals or even amorphous material). The size of nanoparticles was reported to be 30 nm using XRD. Since XRD is smaller than SEM, the particles are likely to be polycrystalline and each particle consists of several crystals.

FTIR analysis was used to detect the biological compounds of plants that reduce and stabilize silver ions. Surface functional groups of nanoparticles act as reducers and coatings for nanoparticles. As shown in (fig. 4), the presence of peaks in regions 3471.74 and 3418.02 may indicate the OH functional group or phenolic compounds. The peak of 1637.43 cm^{-1} can be related to C = O, the peak of 1618.89 cm^{-1} is related to NH2, the peak of 2005 and 1322 cm^{-1} is related to Alkane C-H or CH3 and aromatic groups. peak 1095 is related to C-O-C, peak 776 cm^{-1} is related to PO and 620 cm^{-1} is related to Amide N-H.

DLS method was used to measurement the size of hydrodynamic particles and also to check the size distribution of dispersed particles in a suspension. The findings obtained from DLS analysis showed that the hydrodynamic size of the biosynthesized nanoparticles was approximately 70 nm and the polydispersity index (PDI) was 0.25.

Scanning electron microscope (SEM) is actually a type of electron microscope that this technique is a shape of up-resolution surface imaging. SEM analysis scans the test sample with a focused electron beam to create a high-resolution image of its surface that contains information about the surface topography and composition of the sample. FESEM was utilize to analyze the morphology of Ag NPs, including their shape, size, polydispersity and monodispersed. The nanoparticles synthesized in this research are mainly spherical with a diameter of 40 ± 5 nm. (Fig. 5).

To analyze the constituent elements of biosynthesized silver nanoparticles, EDX spectrum was obtained from the solution **.**Our results showed the formation compounds and relative percentage of compounds in the mixture containing nanoparticles (Fig.6). Silver is the main composition of silver nanoparticles. In addition to that also, there are other elements such as oxygen, carbon, sulfur and nitrogen in lesser quantities in the nanoparticle structure.

3.3. Antibacterial assay

In this study, after characterization of the produced silver nanoparticles, their antibacterial activity against *Pseudomonas aeruginosa, Salmonella typhimurium, Escherichia coli, Staphylococcus aureus* was investigated. According to the obtained results, green synthesized AgNPs showed a significant antimicrobial effect on gram-negative and gram-positive bacteria.

3.3.1. Disk diffusion test

According to the results of the disc diffusion method, the synthesized nanoparticles in all concentrations had an inhibition zone against the tested bacteria, while the extract did not show any measurable growth inhibition zone. The maximum diameter of the inhibition zone was measured for all bacteria at a concentration of 500 ppm (Fig. 7). The diameter of the inhibition zone of different concentrations of silver nanoparticles on the tested bacteria is shown in (Table 1). The maximum diameter of the inhibition zone was measured for *Staphylococcus aureus*, which was 41.5 mm, and the lowest was for *Pseudomonas aeruginosa*, which was measured at 24 mm.

3.3.2. Minimum inhibitory concentration (MIC)

MIC of synthesized silver nanoparticles was measured using microdilution method. The average absorbance of the nanoparticles was calculated by determining the difference between the initial and final absorbance in the wells, and the absorbance values of the silver nanoparticles for the four bacterial species are presented in (Table 2). Based on the results and conducted analyses, no bacterial growth was observed for *Salmonella typhimurium* at concentrations ranging from 500 to 15.7 ppm, indicating that silver nanoparticles inhibited bacterial growth. The lowest concentration at which no turbidity was observed was considered the MIC.A concentration of 15.7 ppm was confirmed as the MIC for *Salmonella Typhimurium*. After determining the MIC, the MIC well (15.7 µg/mL) along with two wells before and two wells after it were cultured. No bacterial growth was observed at 31.25 µg/mL, which was confirmed as the Minimum Bactericidal Concentration (MBC) for *Salmonella typhimurium*. Similarly, *Pseudomonas aeruginosa* was also very sensitive to silver nanoparticles with MIC 31.25 µg/mL and MBC 62.5 µg/mL. *Escherichia coli* and *Staphylococcus aureus* with MIC 62.5 µg/mL and MBC 125 µg/mL showed sensitivity to synthesized silver nanoparticles. The results of one-way variance between *Pseudomonas aeruginosa, Salmonella typhimurium, Escherichia coli, Staphylococcus aureus* at different concentrations of AgNPs confirm that there was a significant difference among different groups and at all concentrations, the bacterial growth was significantly inhibited compared to the control group. The Scheffe post hoc test was utilize to assess which groups were different from the other groups. Significance level in this study was considered as $p \leq 0.05$ (Table 3).

Table 1. Zone of inhibition (mm) of silver nanoparticles against the tested bacteria.

P	Zone of inhibition (mm) Mean ± SD	*bacteria* species
		Ecoli
	32.5 ± 3.535^{b-g}	Cephallexin
	11.5 ± 0.7071$^{a,f-h}$	7/8
	12.5 ± 0.7071$^{a,f-h}$	15/7
0.0001	14.5 ± 0.7071^{a,g,h}	31/25
	16. ± 1.414^{a,g,h}	62/5
	18.5 ± 2.121$^{a-c,g,h}$	125
	27.5 ± 3.532$^{a,-,h}$	250
	37. ± 1.414^{b-g}	500
		S.aurous
	40.5 ± 0.7071^{b-h}	Cephallexin
	13.5 ± 0.7071$^{a,-h}$	7/8
	14.5 ± 0.7071$^{a,-,h}$	15/7
0.0001	16.5 ±0.7071$^{,f-h}$	31/25
	16.5 ± 0.7071$^{a,f-h}$	62/5
	22.5 ±3.535^{a-h}	125
	33.5 ±2.121^{a-h}	250
	41.5 ±2.121^{b-g}	500
		S.tiphymurium
	25 ± 0. 0000$^{b-d,h}$	Cephallexin
	9.5 ± 0.7071$^{a,d-h}$	7/8
	10 ± 0. 0000^{a,d,e,f,g,h}	15/7
0.0001	14.5 ± 0.7071$^{a-c,f-h}$	31/25
	15.5 ± 0.7071$^{a-c,f-h}$	62/5
	24 ± 1.414$^{b-e,g,h}$	125
	26 ±0.7071$^{b-e,f,h}$	250
	28.5 ± 0.7071^{a-g}	500
		P.aeruginosa
	25 ± 0.0000^{b-g}	Cephallexin
	8.5 ± 0.7071$^{a,e-h}$	7/8
	9 ± 1.414$^{a,e-h}$	15/7
0.0002	10 ± 0. 0000 $^{a,e-h}$	31/25
	14 ± 1.414^{a-g}	62/5
	16 ± 2.828$^{a-d,h}$	125
	19 ± 1.414$^{a-e,h}$	250
	24 ± 1.414^{b-g}	500

(a, b, c, d, e, f, g, h values with significant difference) The values are expressed as the mean ± standard deviation. **a:** Compared to the cephallexin **b:** Compared to group 7.8 **c:** Compared to group15.7 **d:** Compared to group31.25 **e:** Compared to group 62.5 **f:** Compared to group125 **g:** Compared to group250 **h:** Compared to group500.

Table 2. Mean and standard deviation Adsorption of microplate wells containing bacterial species in the presence of different concentrations of silver nanoparticles

Bacteria species				Concentration (ppm)
Pseudomonas aeruginosa	***Salmonella typhimurium***	***Escherichia coli***	***Staphylococcus aureu***	
۰.192± 0.009$^{b\text{-}h}$	۰.114± 0.004$^{b\text{-}h}$	۰.231 ± 0.002$^{b\text{-}h}$	۰.142 ± 0.003$^{b\text{-}h}$	0
۰.097 ± 0.002$^{a\text{-}h}$	۰.052 ±0.002$^{a\text{-}h}$	۰.132 ± 0.005$^{a\text{-}h}$	۰.109 ± 0.002$^{a\text{-}h}$	7
۰.050± 0.003$^{a\text{-}b,\ e\text{-}h}$	۰.034± 0.006^{ah}	۰.083 ± 0.002$^{a\text{-}b,\ e\text{-}h}$	۰.084 ±0.004$^{b\text{-}h}$	15
۰.031 ± 0.001$^{a\text{-}b,\ e\text{-}h}$	-۰.007 ± 0.0028$^{a\text{-}c,\ f\text{-}h}$	۰.067 ± 0.006$^{a\text{-}b,\ e\text{-}h}$	۰.049 ± 0.008$^{a\text{-}c,\ f\text{-}h}$	31
-۰.002± 0.001$^{a\text{-}d,\ h}$	-۰.014 ± 0.002$^{a\text{--}d,\ g,\ h}$	۰.028 ± 0.002$^{a\text{-}h}$	۰.036 ± 0.007$^{a\text{-}c,\ f\text{-}h}$	62
-۰.005 ± 0.007$^{a\text{-}d,\ h}$	-۰.020 ± 0.002$^{a\text{-}d,\ h}$	-۰.011 ± 0.002$^{a\text{-}e}$	0 ± 0$^{a\text{-}e,\ h}$	125
-۰.075±0.007$^{a\text{-}h}$	-۰.034 ± 0.002$^{a\text{-}e}$	-۰.021 ± 0.0021$^{a\text{-}e}$	-۰.021 ± 0.001$^{a\text{-}e,\ h}$	250
-۰.011 ± 0.001$^{a\text{-}d,\ g}$	-۰.042 ± 0.002$^{a\text{-}f}$	-۰.0475 ± 0.0021$^{a\text{-}e}$	-۰.060 ± 0.0007$^{a\text{-}g}$	500

(a, b, c, d, e, f, g, h values with significant difference) The values are expressed as the mean ± standard deviation. **a:** Compared to the control group **b:** Compared to group 7.8 **c:** Compared to group15.7 **d:** Compared to group31.25 **e:** Compared to group 62.5 **f:** Compared to group125 **g:** Compared to group250 **h:** Compared to group500.

Table 3. Comparison of the average growth of bacterial species in different concentrations of silver nanoparticles

Mean ± SD					Concentration (ppm)
P	***Pseudomonas aeruginosa***	***Salmonella typhimurium***	***Escherichia coli***	***Staphylococcus aureus***	
.0001۰	.192± 0.009^{abc}۰	.114 ± 0 .004^{abd}۰	.231 ± 0.002^{acd}۰	.142 ± 0.003^{bcd}۰	0
.0001۰	.097 ±0 .002^{abc}۰	.052 ± 0 .002^{abd}۰	.132 ± 0.005^{ac}۰	0.109 ± 0.002^{bcd}	7/8
.001۰	.050± 0.003^{ab}۰	.034± 0 .006^{ab}۰	.083 ± 0.002^{cd}۰	0.084 ± 0.004^{cd}	15/7
.0001۰	.031 ± 0.001^{abc}۰	-۰.007 ± 0.0028^{abd}	.067 ± 0.006^{cd}۰	0.049 ± 0.008^{c}	31/25
.002۰	-0.002±0 .001^{ab}	-0.014 ± 0 .002^{ab}	.028 ± 0.002^{cd}۰	.036 ± 0.007^{cd}۰	62/5
.50۰	-۰.005 ± 0.007	-0.020 ± 0 .002	-0.011 ± 0.002^{d}	0 ± 0	125
.0001۰	-۰.075±0.007^{ab}	-0.034 ± 0.002^{abd}	-0.021 ± 0.0021^{d}	-0.021 ± 0.001^{d}	250
.0001۰	-۰.011 ± 0.001^{abc}	-0.042 ± 0.002^{abd}	-0.0475 ± 0.0021^{acd}	-0.060 ± 0.0007^{bcd}	500

a: Compared with *Staphylococcus aureu* **b:** Compared with *Escherichia coli* **c:** Compared with *Salmonella typhimurium* **d:** Compared with *Pseudomonas aeruginosa* (a, b, c, d Values with significant difference) The values are expressed as mean ± standard deviation (ANOVA: ($p \leq 0.05$).

Figure 1. Colour change in the extract and silver nitrate after incubation

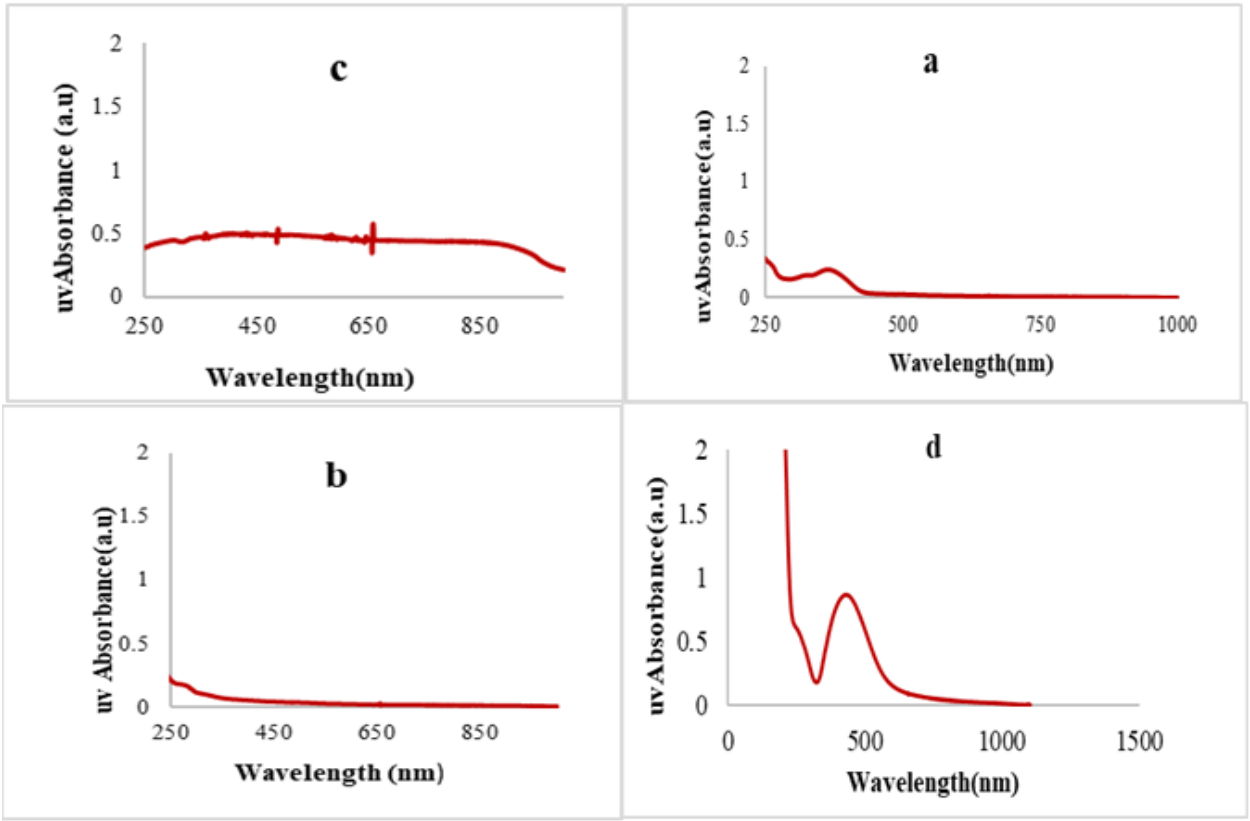

Figure 2. UV–Vis absorptionspectra of synthesized silver nanoparticles **a:** *Peganum harmala* extract **b:** *Allium sativum* extract **c:** Silver nitratesalt **d:** silver nanoparticle (Ag NP)

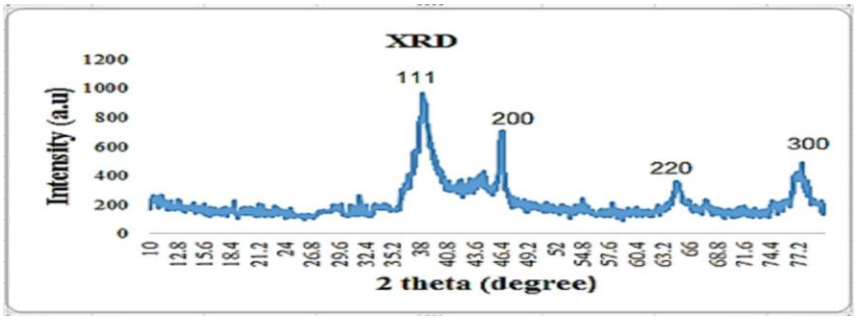

Figure 3. XRD Pattern of synthesized silver nanoparticles.

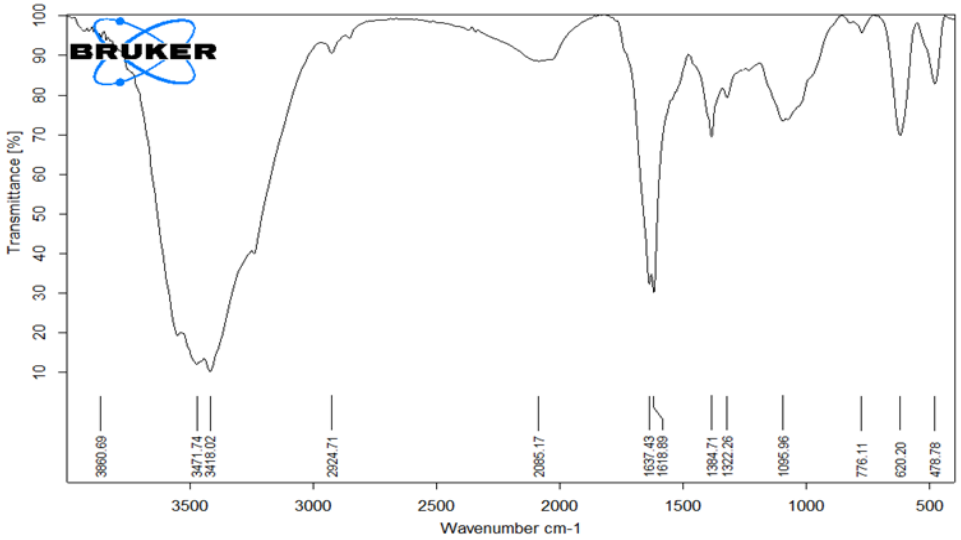

Figure 4. FTIR spectra of synthesized silver nanoparticles.

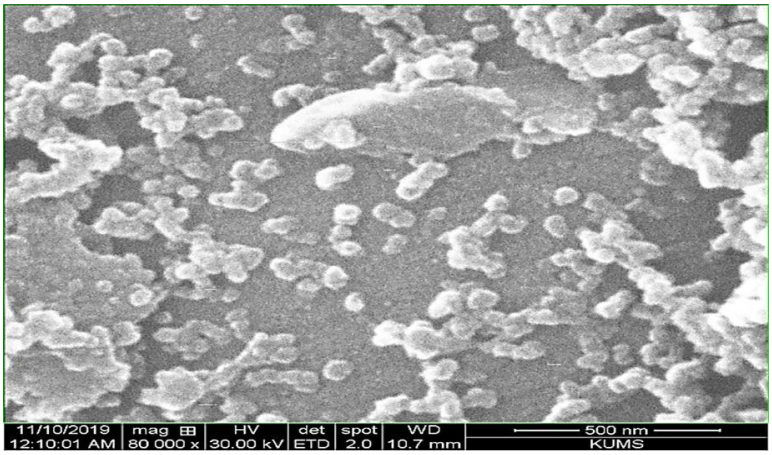

Figure 5. SEM image of synthesized silver nanoparticles.

Concentration(%W/W)	Element
60.22	Ag
9.37	O
21.90	C
5.36	N
3.41	S

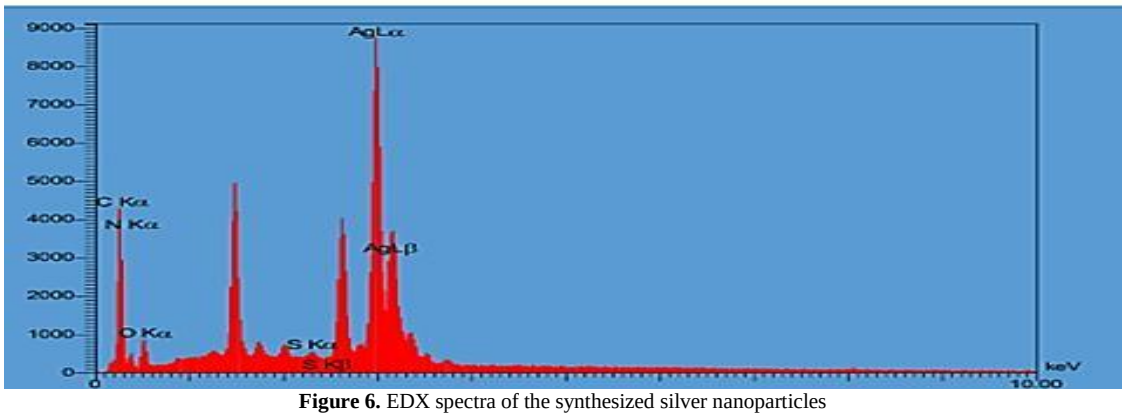

Figure 6. EDX spectra of the synthesized silver nanoparticles

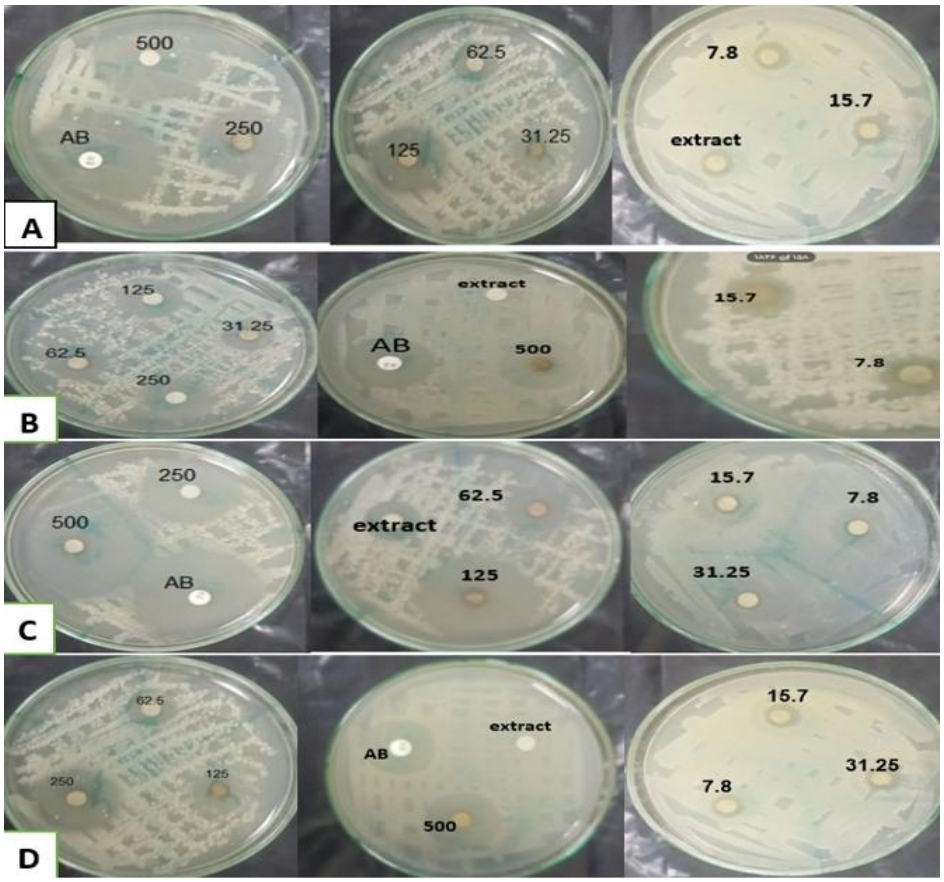

Figure 7. Inhibition zone (mm) of silver nanoparticles against tested bacteria**. A:** *Escherichia coli* **B:** *Pseudomonas aeruginosa* **C:** *Staphylococcus aureu* **D:** *Salmonella typhimurium*

4. Discussion

Considering the spread of drug resistance, nanoparticles can be a promising solution to deal with drug resistance. Because they can not only be used as antibacterial compounds, but also in the field of drug delivery and drug transportation. In the green synthesis method, microorganisms and plants are used, which are compatible with the environment. Plant extracts, due to their antioxidant properties and many secondary compounds, have the role of reducing and stabilizing nanoparticles. So far, AgNPs have been Biosynthesis with extracts of different plants(Jelinkova et al., 2019). However, in this research, for the first time, AgNPs were biosynthesized utilize the combined extract of two plants *Allium sativum* and *Peganum harmala*, and its Antibacterial effects were investigated.

Nanoparticles have attracted attention in various fields due to their unique properties such as high contact surface, high light scattering and mechanical properties. The optical properties of nanoparticles change according to the size and shape of nanoparticles, which is very important in the effect of nanoparticles. As shown in Figure 1, The Discoloration of the solution is the first sign of the synthesis of nanoparticles, which is related to the reduction of Ag+ to AgNPs. According to previous reports, the color change is caused by the excitation of surface plasmon vibrations (Ghaderi et al., 2020). When metal nanoparticles are placed in the range of UV wavelength radiation, according to the theory of surface plasmon resonance, they are excited in a specific length and will have an absorption maximum. Factuality, the free electrons of AgNPs is excited, but because the electron is unstable in the excited state, it returns to the initial energy level and emits a photon. In previous studies, it has been reported that the SPR of AgNPs is in the range of 400 to 450 nm, and in the present study, it was observed in the peak range of 450 nm (Veerasamy et al., 2011). The XRD pattern clearly confirmed the formation of silver nanoparticles. XRD analysis revealed distinct peaks at 4 values of 2θ that can be attributed to the 111, 200, 220 and 311 crystal planes of AgNPs. According to this model, AgNPs with a high degree of purity were biosynthesized. The obtained XRD patterns are consistent with previous studies (Rakib-Uz-Zaman et al., 2022). In the FTIR diagram, there are several peaks that indicate the presence of functional groups such as OH, C=O, NH2, C-H, PO and C-O-C on the surface of silver nanoparticles. The presence of these bands indicating the formation of silver nanoparticles coated with biomolecules which confirms that compounds such as flavonoids, alkaloids and aromatic compounds present in the extract an effective task in the reduction and biosynthesis of AgNP, which are in line with the results of other researchers (Renuka et al., 2020; Singh et al., 2021). DLS technique was used to measure the size of nanoparticles and their dispersion distribution. As the results showed, the diameter of silver nanoparticles produced was between 70 and 80 nm, with a PDI of 0.25, which This value of PDI confirms the uniformity of the biosynthesized AgNPs (Tanase et al., 2020). FESEM was utilized to analyze the morphology of Ag NPs, including their shape, size, polydispersity and monodispersed. The nanoparticles synthesized in this research are mainly spherical with a diameter of 40 ± 5 nm. The shape and size of nanoparticles depend on salt concentration, amount of extract, type of extract, duration of reaction and environmental conditions (Singh et al., 2015). EDX spectrum was used for elemental analysis of biosynthesized silver nanoparticles. It is widely known that metallic silver nanoparticles typically exhibit an absorption peak of nearly 3 keV. Upon analyzing the EDAX graph, the presence of element silver can be detected in the graph procure of EDAX Technique and corroborates the findings of the XRD results. This suggests the reduce of Ag+ to element Ag. As shown in Figure 6, there are 60.22 elements of silver in the sample, and elements such as oxygen, carbon, sulfur, and nitrogen, which are related to the coating molecules, are present to a lesser extent. which are in line with the results of other researchers (Al-Otibi et al., 2021; Hemlata et al., 2020).

The results obtained from this research showed that the biosynthesized nanoparticles have a very high antimicrobial effect on all the selected bacteria. The antimicrobial effect of AgNPs biosynthesized with the combined extract of two *Allium sativum* and *Peganum harmala* plants shows that it more effectively prevents the growth of Gram-negative bacteria,

especially *Salmonella Typhimurium*. This antimicrobial effect of silver nanoparticles is consistent with findings from other studies, which have demonstrated that silver nanoparticles are capable of inhibiting bacterial growth, particularly against Gram-negative bacteria This antimicrobial effect is likely due to the release of silver ions from the nanoparticles, which damage the bacterial biological membrane, leading to increased membrane permeability and ultimately cell death (Dibrov et al., 2002; Joshi et al., 2018; Khan et al., 2017; Kharat & Mendhulkar, 2016; Shah et al., 2020; Singh et al., 2021; Su et al., 2015). The antimicrobial effect of *Allium sativum* and pecan plant extracts has been investigated separately in some other researches. Garlic extract is known for its presence of sulfur compounds such as allicin, which has strong antimicrobial properties. Several studies have shown that garlic extract has a significant effect, especially against Gram-negative bacteria such as E. coli and *Salmonella typhimurium* (Bhatwalkar et al., 2021). In addition, *Peganum harmala* extract, which contains alkaloid compounds, also has antimicrobial effects on bacteria and fungi. Research has shown that pecan extract can be effective in inhibiting the growth of bacteria and fungi (Khadraoui et al., 2022). Comparing the antimicrobial effect of combined extract of garlic and pecan with silver nanoparticles, the results showed that silver nanoparticles have significantly higher effectiveness in inhibiting the growth of bacteria than combined plant extracts. This result is consistent with previous research that confirmed the greater effect of (Singh & Mijakovic, 2022). Due to their unique physicochemical properties, such as small size and high specific surface area, silver nanoparticles can bind more effectively to bacterial membranes and destroy them from the inside (Le Ouay & Stellacci, 2015). Due to their small size, silver nanoparticles create more contact surface with bacteria and allow the silver ions released from them to directly bind to the biological membranes of bacteria and disrupt cellular activities. These ions are able to penetrate bacteria and damage the cell membrane, destroy internal structures, and increase membrane permeability, which ultimately leads to cell death (Tang & Zheng, 2018). On the other hand, it was observed that (Prasad et al., 2017) This difference in sensitivity can be attributed to the distinct structural composition of their cell membranes. The cell membrane of Gram-negative bacteria comprises a thin peptidoglycan layer and an outer phospholipid membrane containing proteins and lipopolysaccharides(Prasad et al., 2017). The outer membrane can act as a barrier to antimicrobial agents to some extent. However, silver nanoparticles, due to their small size and high surface area, can efficiently penetrate this membrane and cause damage. Silver ions (Ag+) easily bind to the lipid structures of the outer membrane in Gram-negative bacteria, leading to membrane disruption and increased permeability (Rajeshkumar, 2016).

Based on the results obtained in this research, the antibacterial effect of synthesized nanoparticles depends on the concentration of nanoparticles, so that with the increase of the concentration of nanoparticles, the antibacterial effect increases strongly, which was similar to the results of other researches (Singh et al., 2021). Antimicrobial performance of nanoparticles on bacterial cells effectively depends on physicochemical properties such as surface morphology and size of nanoparticles. Previous research has shown that the size of a nanoparticle plays a critical role in its antibacterial effect (Pal et al., 2007). Smaller NPs have a more specific surface area and therefore the probability of contacting and passing through the cell membrane of bacteria is higher (Shah et al., 2020). The biosynthesized nanoparticles in this study also have a spherical shape and a size of 40 to 45 nm, which according to the results have significant antimicrobial effects and it was consistent with the results of other researches (Deplanche et al., 2010). In general, the results of this research showed that biosynthesis silver nanoparticles have a very high antimicrobial effect due to their specific synergistic and physicochemical properties. These findings highlight silver nanoparticles as an innovative antimicrobial agent and can show that these nanoparticles synthesized with plant extracts can be used as a sustainable agent in the fight against antibiotic-resistant bacteria and other pathogens.

Conclusion

Based on the findings of this study, we conclude that the green synthesis method, utilizing *Allium sativum* and *Peganum harmala* extracts, is an effective and environmentally friendly approach for synthesizing silver nanoparticles (AgNPs). The synthesized AgNPs, characterized by techniques such as DLS, XRD, FTIR, SEM, and EDX, were spherical in shape with an average size of 40 ± 5 nm. The nanoparticles demonstrated significant antibacterial activity against a range of pathogens, including *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhimurium, and Escherichia coli*. The minimum inhibitory concentrations (MICs) were 62.5 ppm for *S. aureus* and *E. coli*, 15.7 ppm for *P. aeruginosa*, and 31.25 ppm for *S. Typhimurium*. These results suggest that the green-synthesized AgNPs possess strong antimicrobial properties, making them a promising candidate for further research and potential applications in antimicrobial therapies.

Refereces

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