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RESEARCH ARTICLE

Eco-Friendly Synthesis of Silver Nanoparticles Using Prosopis farcta Fruit Extract and Evaluate Their Biological Applications

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ABSTRACT

Recent research has shown increased interest in silver nanoparticles (AgNPs) research due to their promising applications. Since that most bacterial species became resistant to antibiotics that are available locally, they pose a serious threat to healthcare institutions. Thus, the creation of a substitute tool for the management of multi-resistance bacteria becomes imperative. Hence, this paper describes a fast and environmentally friendly method for synthesizing silver nanoparticles (AgPFNPs) using the aqueous solutions of P. Farcta extract and assessing their antimicrobial efficacy against pathogenic bacteria.

The GC-MS measurements were carried out to analyze the Prosopis farcta extract's chemical composition. Different The AgPFNPs' absorption band location was scanned using an ultraviolet-visible (UV-vis) spectrophotometer and the Fourier-transform infrared spectroscopy (FTIR) was used to reveal the biomolecules in the plant extract. The size distribution and images of AgPFNPs were investigated using a Zetasizer and scanning electron microscopy (SEM) measurements, respectively. The antibacterial activity of AgPFNPs was evaluated against two types of pathogenic bacteria.

The results revealed that P. farcta fruit has eleven phytochemical components discovered in it. The major component was Ethyl. Alpha.-d-glucopyranoside (81.0146 %), while, Catechol was the lower one (1.0296%). In addition, the changing of the reaction mixture's colour from pale yellow to dark brown was an indication of AgPFNP formation. AgPFNPs recorded an absorbance peak at 436 nm and had a proper size of 42.71 (d. nm) and a Z-average of 102 (d. nm). FTIR examination revealed the types of chemical combinations in the P. farcta extract that play a role in the production and surface capping of AgPFNPs. SEM images demonstrated the structure of dispersed AgNPs on the nanoscale. Moreover, AgPFNPs displayed full hemocompatibility with RBCs and exhibited a distinctive antibacterial activity with inhibition zones of 11.77±0.18 mm and 14.07±0.29 mm against Staphylococcus aureus and Escherichia coli, respectively.

INTRODUCTION

Nanotechnology is an interdisciplinary science, covers a wide and diverse range of sciences of engineering, biology, physics, and chemistry [1]. Nanoparticles and nanomaterial applications are expanding rapidly due to exceptional properties in particular particle size, large surface area, high

chemical stability, surface reactivity, charge, and shape relative to their raw materials [2]. When the dimensions of a material are reduced to less than 100 nm, powerful changes in the physical and chemical properties can occur [3].

Silver metal, one of the most widely utilised materials in a variety of uses, was first employed as an antibacterial and subsequently as an anti-inflammatory agent to treat some illnesses [4]. Metal nanoparticles are the attractive focus of rapidly expanding research, due to new properties of particle size, large surface area, high chemical stability, surface reactivity, charge, and shape relative to their raw materials, which lead to a vast scope of applications [5].

Silver nanoparticles, referred also as AgNPs, are small particles that range in size from one to one hundred nanometres. Compared to bulk metals, they have strong variances in their physical and chemical features, giving them special characteristics that depend on their size, shape, and dispersion [6]. Nanostructured silver particles are seen as more appealing materials with potential uses in a variety of industries, including food packaging [7], bioremediation and catalysis [8], and most recently, widespread usage as antibacterial agents against harmful bacteria and fungus [9]. Seeing pathogenic bacteria develop their resistance to locally available antibiotics [10], the demand for effective antibacterial tools continues. Although AgNPs as antimicrobial agents are still debatable, research into employing AgNPs to limit the causes of infections is ongoing [11], since the effects of the silver ions that AgNPs produce outweigh what is known about them [12]. Many investigations have documented that the releasing of silver ions, subsequently, disrupts membrane permeable, causing cell components to leak. According to [13], silver ions cause bacterial mortality by promoting reactive oxygen species (ROS), which break cell walls.

Silver nanoparticles can be prepared utilising several ways, such as physical, chemical and biological. In chemical procedures, metal ions are reduced to nanoscale particles by chemical reducing agents [14]. Nonetheless, there is an increasing demand to synthesise nanoparticles using environmentally benign methods [15]. To create nanoparticles with an appropriate size, in a single step, and with less toxicity than chemical production methods, Eco-friendly technologies, utilising plants, fungi, algae, and microbes, are favoured over other methods [16].

Plant extracts from variety of plant parts such as leaves, stems, and roots including garlic, peppers, and eucalyptus, have been shown to have potent antibacterial properties [17]. Hence, in this study, *Prosopis farcta* (*P. farcta*) was selected as a medicinal plant for synthesizing and capping AgNPs nanoparticles with antimicrobial effectiveness and an available option for pathogen management. *P. farcta* (Banks ET Soland) Macbride belongs to the Fabaceae (Leguminosae) family, commonly known as the mesquite or the Aleppo mesquite. Its original habitats are western parts of Asia and spread to all Mediterranean area, South Africa It is native to the Middle East and is known for its ability to thrive in arid and semi-arid environments [18]. Particularly, *P. farcta* has a wide range of chemical substances, including tannins, alkaloids, quinones, phenolics, and flavonoids. It is true that *Prosopis* plants are rich in phenolic compounds; the most prevalent ones are anthocyanins and the flavonoids luteolin, quercetin, apigenin, and their derivatives [19].

Among other conditions, the Prosopis genus has historically been used to treat asthma, callouses, conjunctivitis, diabetes, diarrhea, expectorant, fever, flu, lactation, liver infection, malaria, otitis media, pediculosis, rheumatism, scabies [20]. The tree can be used for wood, food, and fodder, among other things. Additionally, *P. farcta* can be utilized to treat cardiovascular diseases, skin inflammations, spasm, stomach ache, and the removal of pancreatic and bladder stones [21]. As well as Prosopis plants have a variety of bioactive qualities, including antioxidant, antibacterial, anticancer, and antidiabetic activities [22].

It was suggested to employ the aqueous extract of *P. farcta* fruits as an agent for AgNPs constructing and stabilizing in a facile, rapid, and cost-effective synthesis procedure over 1 hour of reaction time at room temperature. Furthermore, our study is displayed as the first to examine the antibacterial properties of AgNPs generated from *P. farcta* fruit extract. The study's schematic is displayed in Fig 1.

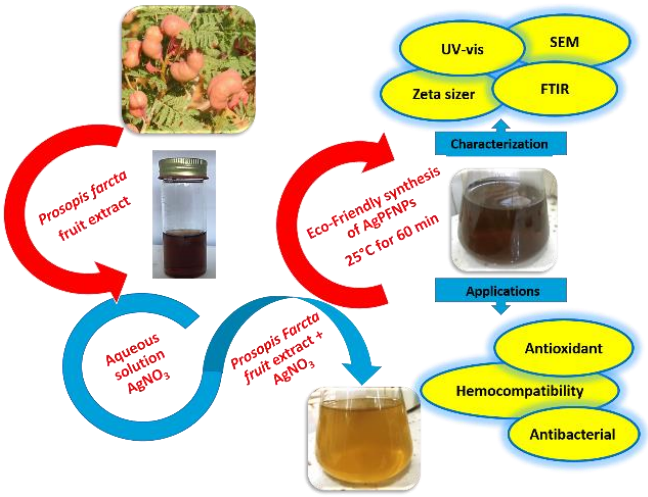

Figure 1: A schematic of the eco-friendly synthesis of AgPFNPs, characterization and applications

MATERIALS AND METHODS

Materials

The following materials were purchased from Sigma Aldrich and used in the study: ascorbic acid, silver nitrate (99%), Muller Hinton agar, Sabouraud dextrose agar, and nutritional broth. While the materials: 2, 2-diphenyl-1-picrylhydrazyl (DPPH) 97%, methyl alcohol, and ethyl alcohol were bought from Fisher Scientific. Additionally, S. aureus and E. coli, were the bacterial isolates that were employed.

Methods

P. farcta fruits collection and its chemical compounds identification

P. farcta were collected in 2023 from various locations inside Basrah, Iraq, and were air-dried before being ground in a Moulinex mill. The powdered fruits (60 g) were extracted using 500 ml of ethanol extract (70% v/v) by Soxhlet apparatus for 48 hours. The extracts were concentrated with a rotary evaporator.

GC-MS analysis of P. farcta ethanolic extract

GC-MS analysis was performed with an ultra-gas chromatograph, the Shimadzu GC-QP 2010 at Basrah Oil Company / Nahran Omar laboratories, using helium gas as carrier gas. It flows at a velocity of 20.4 cm/s and is helium (0.6 ml/min) and an injection temperature of 250°C. The injection column's temperature was set to 80°C, and it rises in accordance with a heat program at a rate of 4°C/min until the temperature reaches 250°C. The Spectral Library was used to diagnose the curves' spectra (NIST, 2005).

P. farcta aqueous extract preparation

The aqueous extract was used for green synthesis of AgPFNPs nanoparticles, to perform that, P farcta fruit extract was prepared, as described in a method by [23], with minor adjustments. In a glass Erlenmeyer flask (250 ml), 5 gm of dried powder and 100 ml of sterile distilled water were combined, then the mixture was continuously stirred and allowed to sit at room temperature for 1 hours. The mixture was then filtered by Whattman No.1 filter paper. Heavy parts of mixture were removed by a centrifuge at 5,000 rpm for 10 minutes, next, rotary evaporator was used to dry the extract at 40 °C for a full day. Finally, the dry powder was stored at 4 °C until required.

Synthesis of silver nanoparticles (AgPFNPs)

Silver nanoparticles were fabricated with a freshly made aqueous extract of the P. farcta fruit (3%) [24]. Under established reaction conditions, 10 ml of P. farcta fruit aqueous extract and 90 ml of aqueous silver nitrate ($AgNO_3$) (0.001 M) were combined in a sterile 100 ml flask and vigorously stirred at room temperature for 60 min. The colour of the reaction mixture was changed from honey to dark brown, indicating the full reduction of Ag^+ ions to AgNPs.

Characterization of silver nanoparticles (AgPFNPs)

The Surface Plasmon resonance (SPR) band and its location were determined, to verify the synthesis of AgPFNPs, using a UV-Vis spectrophotometer that operated in the 200–800 nm range. AgPFNPs' size and dispersal were analysed using the Malvern Zetasizer Nano ZS. The chemical components of the plant extract and the structure of AgPFNPs were determined by FTIR instrument (BRUKER, University of Basrah), which was carried out at wavelengths between 4000 and 400 cm^{-1} in the mid-IR range. AgPFNPs images were captured using Scanning Electron Microscopy (SEM) (University of Basrah),with magnification of 100,000 times, electron window at 5.3 mm and high-resolution units running at a voltage of 13.00 HV [25].

Hemocompatibility analysis for AgPFNPs with red blood cells

A hemolysis experiment was performed to examine the hemocompatibility of AgPFNPs [26]. In anticoagulant tubes, 10 ml of fresh blood were drawn for the hemolysis test. This test was applied by utilising tween X as the positive control, whereas, sterilized normal saline was the negative control. To perform this analysis, two ml of blood was put in three tubes. The RBCs of all tubes were collected by centrifuging, for 6 min at 10,000 rpm, then were washed with 4 ml of sterilized normal saline and centrifuged for 6 min at 10,000 rpm. After that, for the test sample, 4 ml (50 µg\ml) of AgPFNPs, were combined with two ml of blood. In addition, for the positive and negative control samples, four ml of tween X and sterilised normal saline were separately, combined with two ml of blood. The tubes were then incubated at 37∘C for an hour in a water bath. All tubes were centrifuged for 3 min at 10,000 rpm, and the supernatant was collected and used to measure the absorbance using a plate reader for an enzyme-linked immunosorbent (ELISA) at a wavelength of 545 nm. The percentage of hemolysis was calculated by following the formula:

$$\% \text{ Haemolysis } = \frac{\text{Mean OD of Sample} - \text{Mean OD of negative control}}{\text{Mean OD of positive control} - \text{ Mean OD of negative control } \times 100}$$

Antioxidant activity of AgPFNPs using the DPPH assay

The antioxidant influences of AgPFNPs were analysed using 2, 2-Diphenyl-2-picrylhydrazyl (DPPH) scavenging assay suggested by [27]. Various concentrations of AgPFNPs and ascorbic acid as a positive control (25, 50, 100, 200, 400 and 800 µg/ml) were divided into the test tubes, with one tube for DPPH as a negative control. To conduct the assay, 1 ml of DPPH (1 mM), disbanded in methanol, was mixed with AgPFNPs and ascorbic acid samples, and then the tubes were vortexed thoroughly. The tubes were set in a dark place at 25 C for 30 min, then were performed for absorption at 517 nm using a plate reader for an enzyme-linked immunosorbent (ELISA). The scavenging activity was assessed using the formula: % of antioxidant activity = [(Ac−As) ÷Ac] × 100

Where: Ac = OD of A. A.; As = OD of AgPFNPs [28].

Antibacterial assessment of AgPFNPs and P. farcta aqueous extract

The well diffusion approach to consider AgPFNPs' antibacterial efficacy toward pathogenic isolates of S. aureus and E. coli, was conducted following a method by [29]. Briefly, the bacterial isolates were inoculated in 4 ml of brain heart infusion broth, then incubated at 37°C until they reached a value of absorbance 0.5 compared to the McFarland measures of 1.5×10^8 CFU/ml. To experiment, 100 µl of each bacterial culture was cultured on the Muller-Hinton (MH) agar surface and then allowed to dry. Following, three wells of 6 mm in diameter in each plate were prepared and then individually loaded with 100 µl of plant aqueous extract 3%, $AgNO_3$ (1mM), and AgPFNPs (10 µg\ml). The plates were kept in the incubator overnight at 37°C. The inhibitory zones around wells and the discs of antibiotics were measured and contrasted to the standard tables. Moreover, the antibiotic discs of Norfloxacin (NOR) and Ciprofloxacin (CRO), were used for S. aureus and E. coli as resistant bacteria to these antibiotics [30]. The plates were incubated at 37°C for 24 hours. The inhibition zones' diameters for each well and antibiotic disc were recorded and compared with the standard controls.

RESULTS AND DISCUSSION

GC-MS investigation

The GC-MS analysis of P. farcta fruits revealed the presence of 11phytochemicals compounds (Figure 1 and Table 1). The active principles are shown in their corresponding retention time (RT), chemical formula, and peak area as a percentage % (Table 1). The major components were Ethyl. alpha-d-glucopyranoside (81.0146 %), followed by alpha-Methyl 4-O-methyl-D-mannoside (4.6865 %), n-Hexadecanoic acid or Palmitic acid (2.6483 %), 2(3H)-Furanone, 5-methyl- (2.2569 %), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (1.8282) and Catechol (1.0296%). Our results are consistent with [32] by found Palmitic acid in P. farcta fruit extract.

Table 1: Essential oil compounds in P. farcta using GC-MS

Peak	Chemical compounds	Chemical formula	Retention time	Area %
1	Oxime-, methoxy-phenyl-_	$C_8H_9NO_2$	8.165	0.9378
2	2(3H)-Furanone, 5-methyl-	$C_5H_6O_2$	8.459	2.2569
3	Pentanal, 2,3-dimethyl-	$C_7H_{14}O$	9.851	1.3749
4	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	12.492	1.8282
5	Catechol	$C_6H_4(OH)_2$	13.607	1.0296
6	Phenol, 2,6-dimethoxy-	$C_8H_{10}O_3$	15.525	1.2675
7	Ethyl .alpha.-d-glucopyranoside	$C_8H_{16}O_6$	19.415	81.0146
8	.alpha.-Methyl 4-O-methyl-D-mannoside	$C_8H_{16}O_6$	19.861	4.6865
9	Scyllo-Inositol	$C_6H_{12}O$	21.467	0.9401
10	n-Hexadecanoic acid or Palmitic acid	$C_{16}H_{32}O$	22.413	2.6483
11	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	$C_{16}H_{22}O$	28.962	2.0156
				100.00

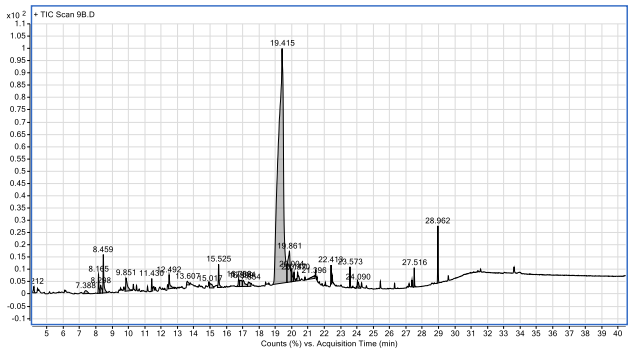

Figure 2: The whole set of P. farcta active compounds utilising GC-MS analysis

Eco-friendly AgPFNPs

Figure 3 shows the colour change of the reaction mixture from pale yellow (Figure 3. a) to dark brown (Figure 3. b) after 60 minutes of incubation at 25°C on a magnetic stirrer.

(b) (a)

Figure 3: Eco-friendly AgPFNPs nanoparticle fabrication (a) Time 0 of (Prosopis. farcta + $AgNO_3$) aqueous solutions incubation, and (b) 60 min post AgNPs fabrication

Figure 3 illustrates how (Ag^1 ions) were reduced to metallic silver (Ag^0), which were then built into small crystals that finally changed into AgNPs. Switching the pale yellow colour of the solution (P farcta fruit extract and silver nitrate), to dark brown hue signifies the AgPFNP synthesis. These findings are comparable to previous statements [33]. In aqueous solutions, the AgNPs exhibit a brown colouration due to the distinctive phenomenon of SPR related to AgNPs [34]. Given that the P. farcta plant contains a variety of biologically active substances (Table 1 and Fig. 2), such as ketones, tannins, proteins, polysaccharides, flavonoids, terpenoids, phenolic acid [19], it has been proposed that the presence of these compounds is what led to the potential fabricate of silver nanoparticles.

AgPFNPs Characterisation

Optical absorbance analysis of AgPFNPs

The optical absorbance statements of AgPFNPs are shown in Fig. 4, where the greatest SPR peak is located at a wavelength of 436 nm. Our findings are consistent with earlier research since it has been shown that green synthesised AgNPs display absorption peaks focused in the vicinity of 420-450 nm [35]. Using a UV–vis spectrophotometer is one of the best approaches to characterise different types of nanoparticles. The absorption band of AgNPs is related to the conductive electrons around the nanoparticles' surface that demonstrate a distinct SPR [22, 36]. Furthermore, the location of AgNPs' absorption band provides information about their size, as smaller AgNPs showing a blue shift in the absorption peak relative to larger AgNPs. As per the study of [37], AgNPs with a size of (15.3 nm) showed an absorption band of (412 nm). The absorbance at 436 nm could indicate that AgPFNPs have a size higher than 15 nm, which would be confirmed by further analysis in this study.

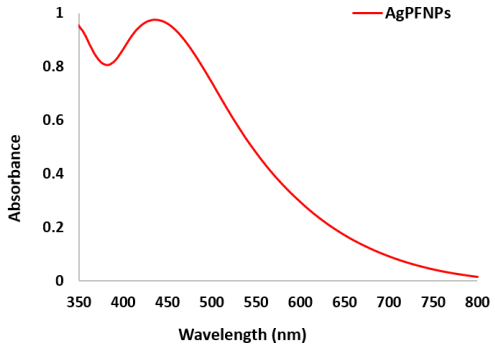

Figure 4: The optical absorbance of silver nanoparticles synthesized with Prosopis farcta extract

AgPFNP Zetasizer test

To determine the size distribution of AgPFNPs nanoparticles, the Zetasizer was involved (Malvern instrument), (Physics department, College of Sciences, Basrah University). The size distribution of AgPFNPs is displayed in Fig. 5. The momentous information is displayed by Zetasizer criteria such as the sample quality, the particle size, and the Standard deviation. The distribution summary yielded a worth of 0.402 for the Polydispersity index (PDI), revealing that AgPFNPs are adequately monodisperse or might have a mid-range value. Table 2 provides a summary of the average size dispersal evaluation. These results are in line with those of previous research [38].

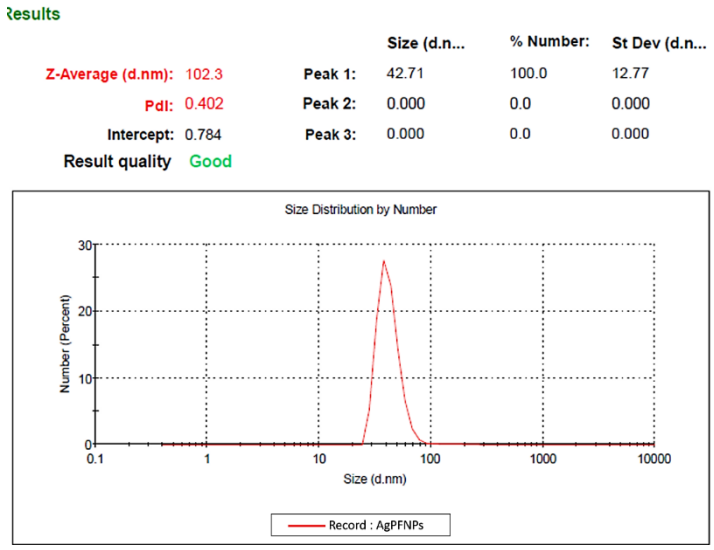

Figure 5: Size distribution of AgPFNPs

Table 2: Report of the size distribution using Zetasizer analysis

Parameter	Value
Z-Average (d.nm)	102.3
PDI	0.402
Size (d.nm)	42.71
% number	100
Standard Deviation	12.77
Intercept	0.784
Result quality	good

Fourier-transform infrared spectroscopy (FTIR) measurements of AgPFNPs

FTIR spectra within the range of 4000-400 cm^{-1} were acquired to determine the influential groups responsible for the AgPFNPs fabrication and stabilisation. The FTIR analysis of eco-friendly AgPFNPs is shown in Fig. 6. A broad, sharp absorption peak has been detected at 3347.90 cm^{-1}, suggesting a hydroxyl group (-OH) in the alcoholic substances of P. farcta extract. Furthermore, three anticipated peaks with wavelengths of 2356.02, 1636.65, and 565.68 cm-1 are observed from the FTIR analysis of AgPFNPs. The three peaks might be due to the interaction of silver (I) ions with P farcta active groups that act as reducing and capping agents to the AgPFNPs. These findings are supported by prior investigations that located identical peaks from green synthesised silver nanoparticles [39].

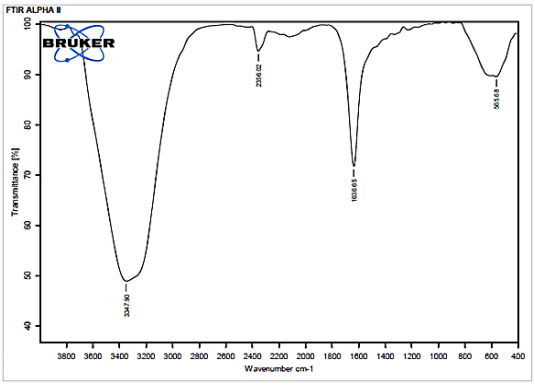

Figure 6: FTIR analysis of P. farcta extract-based synthesised AgPFNPs

Scanning electron microscopy (SEM) measurements of AgPFNPs

Figure 7 represents SEM image of AgPFNPs. The images of AgPFNPs were visualized under scanning electronic microscopy (SEM) (University of Basrah), to acquire a visual manifestation of the AgNPs. SEM image analysis indicates that AgPFNPs had a sphere shape with size at 20-50 nm using a scale bar of 1 µm, and showed entire monodisperses as demonstrated by their structural and morphological characteristics. Four images were taken for AgPFNPs and all revealed that AgPFNPs appeared as homogenous particles without any noteworthy aggregation.

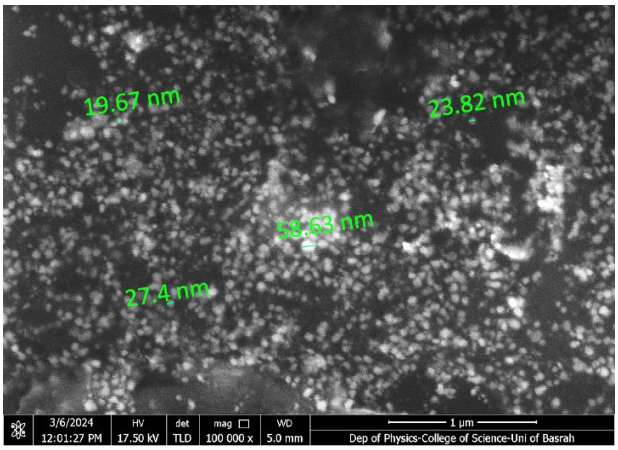

Figure 7: SEM image of AgPFNPs formed by P. farcta fruit extract. Scale bar: 1 µm

Hemocompatibility assessment of AgPFNPs

AgPFNPs produced no haemolytic effects to RBCs (Fig. 8). Besides, haemolysis had not been observed using a normal saline solution. However, the incubation with Triton-X, resulted in a complete haemolysis of 100%. In broad, AgPFNPs were not harmful to RBCs in contrast to Triton-X

[17]. These findings imply that nanoparticles can be utilized for various biological purposes in future research.

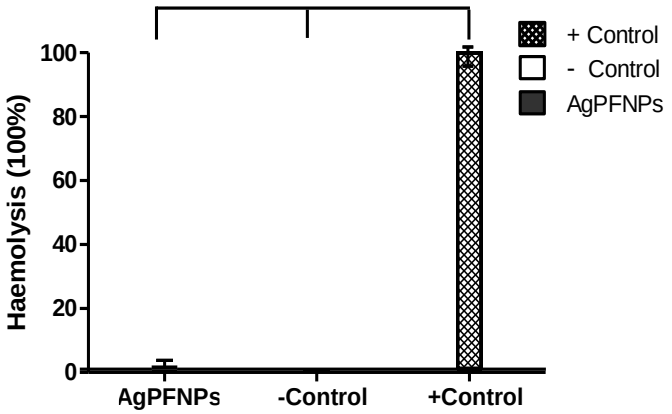

Figure 8: Hemocompatibility analysis of AgPFNPs

DPPH scavenging activity of AgSNNPs

Prosopis farcta extract was utilised for AgPFNP synthesis and capping. The antioxidant activity of AgPFNP was tested utilizing the DPPH scavenging assay [24]. The results indicated that the AgSNNPs were effective as an antioxidant agent relative to the typical antioxidant agent (Ascorbic acid). The dark purple colour of DPPH was decolourized when mixed with The AgPFNPs and ascorbic acid as antioxidant substances, which leads to a change in the absorbance of the DPPH after mixing. The AgPFNPs revealed DPPH scavenging activity of %50 at a concentration of 120 µg/ml compared to the ascorbic acid that offered DPPH scavenging activity of %50 at a concentration of 100 µg/ml.

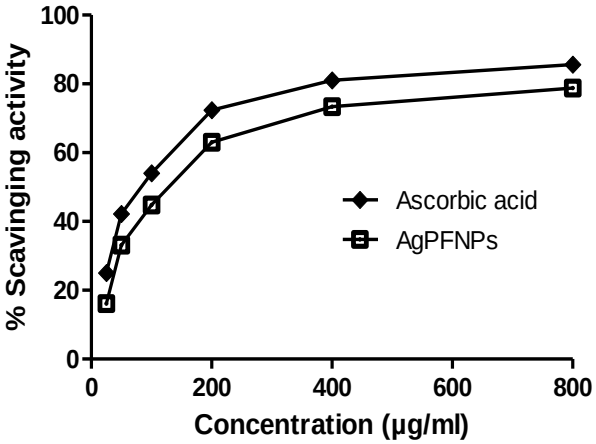

Figure 9: DPPH scavenging activity of AgPFNPs

Antibacterial activities of AgPFNPs

The antibacterial effectiveness of AgPFNPs was evaluated using a well diffusion method by measuring the diameters of inhibition zones around wells against S. aureus and E. coli as Gram-positive and Gram-negative bacteria, respectively. The statistical analysis demonstrated significant differences between S. aureus and E. coli in the measured diameters (Fig 10). As shown in Fig. 10 and Table 3, P. farcta extract had no antibacterial effects on the two types of bacteria when used with concentration 10 mg\ml. While AgPFNPs (4 µg\ml) had antibacterial impacts against both. In addition, $AgNO_3$ had no effect on S. aureus, while exhibited small inhibition zone with diameter of 6 mm against E. coli. The inhibition zones diameters of AgPFNPs around wells were with an average of

11.77±0.18 mm and 14.07±0.29 mm for S. aureus and E. coli, respectively. It is more interested to learn that both isolates were completely resistant to antibiotics used in this study. Our findings are consistent with the investigation of a study that found that P farcta aqueous extract from different parts of plant was not effective compared to antibiotics [40]. Nonetheless, our results align with several pieces of research that have shown distinct inhibitory advantages from AgNPs against MDR pathogenic bacteria [41].

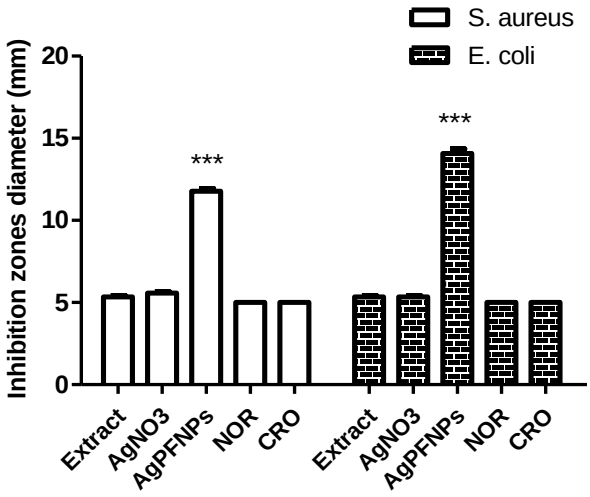

Figure 10: Antibacterial activity of AgPFNPs against S. aureus and E. coli

Table 3: Inhibition zone diameters (mm) against S. aureus and E. coli bacteria

Parameters	**Inhibition zone diameters (mm) against**	
Antibacterial agent	S. aureus	E. coli
P. farcta aqueous extract	5.33±0.08	5.33±0.08
$AgNO_3$ aqueous solution	5.567±0.08	5.423±0.09
Biogenic synthesised AgPFNPs	11.77±0.18	14.07±0.29
Antibiotic of Norfloxacin (NOR)	5.00±0.00	5.00±0.00
Antibiotic of Ciprofloxacin (CRO)	5.00±0.00	5.00±0.00

Overall, these findings support the effectiveness of the chosen synthesis technique for AgPFNPs, which involves reducing $AgNO_3$ by P. farcta fruit extract.

CONCLUSIONS

In the present investigation, the aqueous extract of P. farcta fruits was used as a reducing agent to greatly enhance the synthesis of AgPFNPs safely and ecologically friendly. In addition, characterization analysis of AgPFNPs informs that nanoparticles were effective as antibacterial agents. Moreover, since AgPFNPs are effective against multidrug-resistant pathogenic bacteria, we propose that AgPFNPs should be classified as alternatives to antibiotics and pathogen control agents.

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CONFLICTS OF INTEREST: We attest that each Figure and Table in the manuscript belongs to us. Additionally, authorization for re-publication is linked to images that are not ours. Further, the local scientific commission at the University of Basrah has approved the research under investigation.

AUTHORS' CONTRIBUTION: This work was proposed by I. Albandar, who carried out all the experiments on the synthesis, characterisation, and data analysis of AgNPs. All authors contributed to the manuscript's writing and proofreading.

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