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Biogenic Synthesis of *Solanum nigrum* Mediated Silver Nanoparticles and study its antioxident and Antibacterial Effects

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Abstract: Green nanotechnology has arisen as a feasible choice for the synthesis of different nanoparticles. Silver nanoparticles (AgNPs) are of great interest in recent research, due to their use in a wide range of applications. Multi-resistance pathogens are a major problem in healthcare facilities, due to their ability to develop resistance to locally available antibiotics which leads to difficulties in preventing infectious diseases. Therefore, it becomes an urgent need to develop a novel alternative tool to control multi-resistance pathogens. In this context, this investigation aimed to develop silver nanoparticles (AgSNNPs) by a fast and environmentally friendly method and evaluate their anti-bacterial activity against bacteria that showed multi-resistance to antibiotics. AgSNNPs were phyto-biosynthesised using aqueous silver nitrate $AgNO_3$ (1mM) solution and an aqueous extract of *S. nigrum* (1%). The GC-MS analysis was performed to determine the chemical compounds in the *S. nigrum* alcoholic extract. The ultraviolet–visible (UV-vis) Spectrophotometer was used to view the position of surface plasmon resonance band of AgSNNPs. Fourier-transform infrared spectroscopy (FTIR) was used to identify the types of biomolecules existing in plant extract that are accountable for silver ions (Ag^+) reduction as well as the stability of AgSNNPs. Zeta sizer and scanning electron microscopy (SEM) were used to assess the size distribution and shape of AgSNNPs. The antimicrobial activity of AgSNNPs against two types of pathogenic bacteria, *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) was assessed using the well-diffusion method. The results demonstrated 48 components in the *S. nigrum* ethanolic extract. Linolelaidic acid was the highest one by 24.68%, while phthalate recorded the lowest one (2.98%). The change of mixture colour from light yellowish-green to honey brown after the incubation period validates AgSNNPs synthesis. Phyto-biogenic synthesized AgSNNPs recorded a Surface plasmon resonance (SPR) band at a wavelength of 420 nm and showed a size of 19.26 (d.nm) and a Z-average of 101.2 (d.nm). FTIR analysis indicated the biochemical compounds of *S. nigrum* extract that are implicated in the fabrication and capping processes of AgSNNPs. AgSNNPs were spherical and monodispersed based on a Zeta sizer and FESEM analysis. Further, AgSNNPs showed complete hemocompatibility using blood samples while revealed a distinctive antibacterial activity with inhibition zones of (17.25±1.56), and (15.32±1.27) against *S. aureus* and *E. coli* respectively. It is concluded that AgSNNPs could be used to inhibit antibiotic-resistant pathogenic bacteria with no indicated hemolysis using blood samples.

Keywords: Nanotechnology, Nanoparticles, Silver nanoparticles, Solanum nigrum, Antibacterial

1. Introduction

Lack of production of new antibiotics, during the previous four decades, combined with the tendency of bacterial strains to develop resistance to present antimicrobials, poses a serious threat to individuals' health over the world [1]. Furthermore, owing to the developed ability of pathogens towards common drugs, an alternative medicinal strategy is demanded, which includes new drugs discovery [2, 3]. Commercial drug is usually specific for either bacteria or fungi and are not effective with both, thus, there is a propelled need to find adequate treatment for all pathogens [4]. Moreover, most pathogens can originate biofilms on the biotic surfaces when the presence of suitable conditions, which accordingly leads to developing chronic illnesses [5].

As silver metal is one of the most commonly used materials in different applications, has originally been used as an antibacterial and later has been used for therapeutic purposes as anti-inflammatory agent to heal different diseases [6]. Metal nanoparticles are the attractive focus of rapidly expanding research, due to unique general 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 [7].

Silver Nanoparticles (AgNPs) are particles 1-100 nm in size and possess powerful differences in the physical and chemical properties compared to bulk metals, thus exhibiting unique attributes which rely on size, shape, and distribution [8]. Nanostructured silver particles are considered more attractive materials for promising applications in different fields such as catalysis and bioremediation [9], food wrapping, and recently are vastly used as antimicrobial agents against pathogenic bacteria [10, 11]. Despite the strategies of AgNPs as antimicrobial agents yet contentious, the influences of silver ions released from AgNPs [12], are greater than what is known about AgNPs, thus, investigations regarding using AgNPs to inhibit infections causes are persistent [13]. In earlier studies, it has been reported that released silver ions, later, interfere with membrane permeability leading to cellular components leaking [14]. [15, 16] disclosed that silver ions exert their toxicity by stimulating reactive oxygen species (ROS) that are responsible for cell wall damage, or disturbed the respiratory chain enzymes for nucleic acids and cell walls, thereafter inducing bacterial death.

Various approaches have been used to produce AgNPs, including physical, chemical, and biological (phyto and microbial approaches). However, there is a growing need for environment-friendly methods to synthesize nanoparticles as they do not require instruments and/or bring further hazards from using chemicals [17]. It has been reported that when particular metal nanoparticles are coated by plant extracts [18], or purified secondary metabolites [19], stimulate more antimicrobial effects.

Wild plants utilising is part of the cultural origins of different peoples of the world as most of them are edible and enhance health [20]. Wild plants' secondary metabolites display many health-promoting effects, so they operate as anti-oxidants, anti-fungal, anti-bacterial, and wound-therapeutic aids [21-23].

In the investigation of developed opportunities for the control of pathogens, *S. nigrum* was chosen as a medicinal plant with high efficiency against pathogens. *Solanum nigrum*, also referred to as "Black Nightshade" or "wolf grape," is one of the Solanaceae family members, herb or short-lived perennial shrub. The plant, which is native to Southeast Asia, is found practically everywhere in America, India and Africa [24]. *S. nigrum* contains Solanocapsine, Asparagine, Solanine, Lutein, Tannin, Palmitic acid, and linoleic acid [25]. [26] used GC-MS

to analyze phytochemicals from the ethyl acetate fraction of *S. nigrum* and reported finding 1, 2 benzene dicarboxylic acid, diisooctyl ester (95%) and as Bis (2-Ethylhexyl) phthalate (84%) along with 3 hydroxy 4 carboxy 2 methyl 6 pyridine (58%).

S. nigrum is an edible plant and used medicinally for its neuropharmacological, anti-tumor, antioxidant, and cancer chemo-protective qualities. It is also used as a sedative and skin softener and a drop of fruit juice on the hurting tooth to have a pain-relieving effect. In addition, *S. nigrum* can use to treat diseases like liver illness, cancer, diabetes, and renal disease as it contains chemical compounds that act as antioxidants [27]. The extract of *S. nigrum* leaves contained 23 phyto components, the primary ones being ethyl linoleolate, hexacontan, phytol, and palmitic acid. Good antibacterial action against *Escherichia coli*, *Proteus mirabilis*, *Staphylococcus aureus*, *Pseudomonas aerogenosa*, and *Klebsiella pneumonia* was demonstrated by alkaloids isolated from *S. nigrum* leaves [28, 29].

In this study, it was proposed to use the aqueous extract of *S. nigrum* leaves as a reducing and stabilizing agent to reduce Ag^+ ions to Ag^0 in an aqueous silver nitrate solution to synthesize AgSNNPs as antibacterial agent. In addition, we report that this work is the first to study the competence of *S. nigrum* leaf extract-derived AgNPs as an antibacterial substance. Figure 1 shows the schematic of this study.

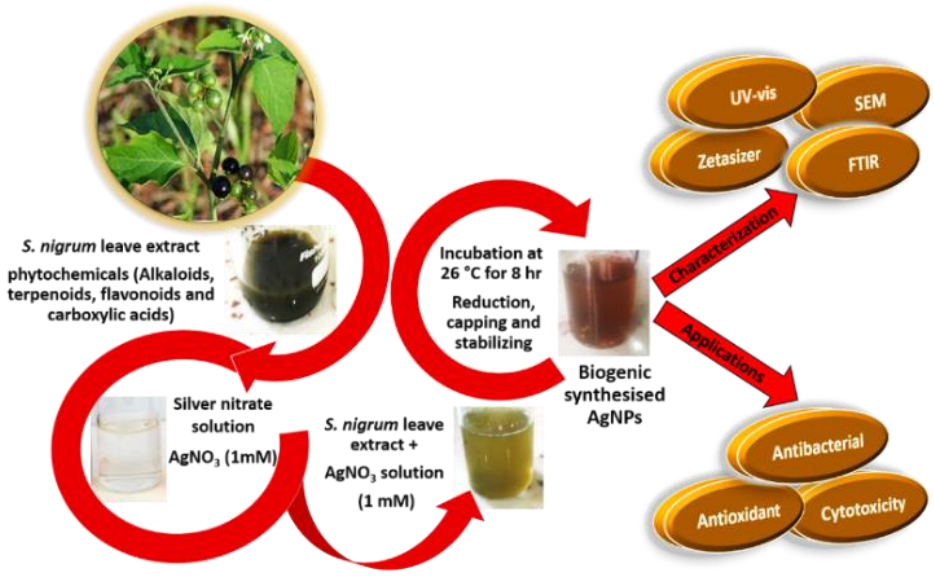


Figure 1. A schematic of the study's themes –Biogenic synthesis of AgSNNPs, characterization and applications

2. Materials and Methods

2.1. Materials.

2.1.1. The chemicals.

The chemicals used were: Brain heart infusion agar, Nutrient agar, Nutrient broth Sabouraud dextrose agar, Muller Hinton agar, Ascorbic acid and Silver nitrate (99%) (Sigma Aldrich). (97%). 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Ethyl alcohol and Methyl alcohol (Fisher Scientific).

2.1.2. Clinical bacterial isolates.

In the current study, *Staphylococcus aureus* and *Escherichia coli* isolates, isolated from clinical samples, skin infections and UTI infections, respectively, were specified for antibacterial investigations. *Staphylococcus aureus* isolates are resistant to Oxacillin (OX), Vancomycin (VA), and Clindamycin (DA). Further, *Escherichia coli* isolates are resistant to Ceftazidime (CAZ), Ampicillin (AM), and Aztreonam (ATM).

2.2 Methods.

2.2.1 Collection of S. nigrum leaves.

S. nigrum leaves were collected from a field in the Basrah Governorate, Iraq, and were washed several times with distilled water to remove the dust and were air-dried at room temperature then stored at room temperature in polyethylene bags until needed. After being pulverized with an electric grinder. The plant samples were kept in airtight glass containers at 4°C until they were used. *S. nigrum* leaves were certified by Prof. Dr. Sahar Abdul Abbas, Department of Biology, College of Sciences, Basrah, Iraq.

2.2.2. Isolation and identification of chemical compounds from S. nigrum leaves.

The samples were extracted by ethanol by taking 25 gm from the ground material and put a 500 ml glass round, add 250 ml of ethanol alcohol. The chemical compounds were separated using gas chromatography-mass spectroscopy analysis.

2.2.3. Diagnosis of chemical compounds in S. nigrum using GC-MS analysis.

Gas chromatography/mass spectrometry (GC-MS) was used to diagnose compounds of *S. nigrum* in the GC-MS laboratories at the Nihran Omer Company, the apparatus type SHIMADZU, Japan, GC MS QP 2010 Ultra, equipped with a capillary column type (DB-MS). Its dimensions are 30 m in length and 0.32 m in diameter, and the thickness of the static phase is 0.25 micrometers. using AcqMethod HP5 ms UI Column 230 C Pulsed splitless. Instrument: 5977A MSD.

2.2.4. Preparation of aqueous S. nigrum leaf extract.

The aqueous extract of *S. nigrum* leaves was processed according to [22], with some modification. The aqueous extract of *S. nigrum* was composed by adding 10 gm of dried powder to 200 ml of sterile distilled water in a glass Erlenmeyer flask (500 ml). The mixture was incubated for 2 hours at room temperature with constant agitation. The extract was acquired by centrifugation at 6,000 rpm for 20 min to remove heavy substances. The filtered extract was then dried using a rotary evaporator at 40 °C for 24 hours and then was stored in the refrigerator at 4 °C until used.

2.2.5. Phyto-biogenic synthesis of silver nanoparticles (AgSNNPs).

AgNPs were synthesized following the protocol of [30]. Succinctly, 4 ml of freshly prepared aqueous extract of *S. nigrum* leaves at a concentration (4%) was added to 96 ml of aqueous silver nitrate ($AgNO_3$) (1×10^{-3} M) in a sterile 250 ml volumetric flask. The flask containing the light yellowish-green mixture was set on a magnetic stirrer hot plate at 26°C

with monitoring the change of color. The complete reduction of Ag^{+} ions to AgNPs was achieved by changing the reaction mixture color from light yellowish-green to honey brown after 8 hr of incubation.

2.3. Characterization of biogenic synthesised AgSNNPs.

The synthesized AgSNNPs were validated by revealing the presence and the position of Surface Plasmon resonance (SPR) band using a UV–Vis spectrophotometer at a range of 200-800 nm. Malvern Zetasizer Nano ZS (v2.2), was used to determine the size distribution of AgSNNPs. All parameters such as Temperature (25°C), Count Rate, Duration Used (s), and Measurement Position (mm) were set. The FTIR analysis of AgSNNPs was performed using FTIR analysis (BRUKER) to identify the chemical composition of the plant and the surface structure of AgSNNPs at the mid-IR region at the wavelengths (4000-400 cm^{-1}). The images of the AgSNNPs were captured using FEI Nova NanoSEM^{TM} 450 Scanning electron microscope (Nebraska Centre for Materials and Nanoscience), with high-resolution imaging – Low voltage 13.00 kV [31].

2.4. Determination of hemocompatibility of AgSNNPs using Red blood cells.

A haemolysis assay was conducted to investigate the cytotoxic impact of AgSNNPs [32]. Fresh blood (10 ml) was collected in anticoagulant tubes for the hemolysis analysis assay. For this assay, sterilized normal saline was used as the negative control and tween X as the positive control. Two ml of fresh anticoagulant blood was mixed with 4 ml of sterilized normal saline for the test sample and 4 ml of distilled water for positive control. The tubes were centrifuged for 10 min at 10,000 rpm, and then the RBCs pellet of the test sample was diluted with 4 ml of sterilized normal saline. Next, different concentrations (10, 20, 30, 40 and 50 µg\ ml), of AgSNNPs, prepared in normal saline, were mixed with samples, and placed in a water bath at 37∘C, for an hour. Each tube was centrifuged at 10,000 rpm for 3 min then the absorbance of the supernatant was registered at 545 nm using an enzyme-linked immunosorbent (ELISA) (800 TS microplate reader). The hemolysis percentage was calculated as follows:

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

2.5. Evolution of Antioxidant activity through DPPH assay.

The antioxidant effects of biogenic synthesized AgSNNPs were investigated using 2, 2-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging potential by the method proposed by [33]. Different concentrations (50, 100, 200, 400, 600, 800 and 1000 µg/ml) of AgSNNPs and ascorbic acid (positive control) were put in separate test tubes, in addition to one tube for DPPH solution (Negative control). To perform this assay, 1 ml of DPPH (1 mM), dissolved in methanol, was added to samples, positive, and negative controls, and then vortexed thoroughly. All tubes were incubated at room temperature in a dark place for 30 min and were utilised for the absorbance examination at 517 nm using a UV–Vis spectrophotometer (UV-1900i-Shimadzu). Free radical scavenging activity was estimated with the formula:

$$\% \text{ of antioxidant activity} = [(Ac - As) \div Ac] \times 100$$

where: Ac = OD of the control; As = OD of AgSNNPs samples [34].

2.6. Antibacterial activity of S. nigrum aqueous extract and AgSNNPs.

The antibacterial activity of AgSNNPs against two pathogenic isolates, *E. coli*, and *S. aureus* was evaluated using a well diffusion method [35]. The isolates were cultured aerobically by streaking on Nutrient Agar (NA) plates, then the plates were incubated at 37 °C for 24 hours. After that, one single colony was inoculated in 5 ml of nutrient broth. The bacterial culture was incubated at 37°C for 60 mins while measuring the absorbance of growth until getting a value of 0.5 of absorbance (identical to the McFarland standards which are equal to 1.5×10^8 CFU/ml). A 100 µl from each bacterial strain culture was spread on the surface of the Muller-Hinton (MH) plate and was then left to dry. Next, three wells in each plate, each one with a diameter of 6 mm, were prepared using a sterile cork borer, then filled with 100 µl of each of *S. nigrum* aqueous solution, $AgNO_3$, and AgSNNPs (10 µg\ml), individually. Furthermore, three types of antibiotic discs Oxacillin (OX), Vancomycin (VA), and Clindamycin (DA), were affixed on the MH plate surface for *S. aureus* as resistant bacteria to these antibiotics. Moreover, another three types of antibiotic discs Ceftazidime (CAZ), Ampicillin (AM), and Aztreonam (ATM) were affixed on the MH plate surface and *E. coli* as resistant bacteria to these antibiotics . The plates were incubated at 37°C for 24 hours. The inhibitory zones around wells and the discs of antibiotics were measured.

3. Results and Discussion

3.1. GC-MS analysis.

GC-MS technology was used to identify *S. nigrum* leaf extract, as detailed in (Table 1) and the main compounds' structures can be seen in Figure 2. The results indicate that 48 components (phytochemical constituents) are present in *S. nigrum* as confirmed by GC-MS analysis. The chemical components from *S. nigrum* leaves are components including (9E,11E)-Octadecadienoic acid (Linolelaidic acid) 24.68%, n-Hexadecanoic acid (Palmitic acid) (14.64%);1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (12.08), 4,6-Di-O-methyl-.alpha.-d-galactose 6.01%; 3-Methylundecanoic acid 5.47% , Eicosane 4.57%, Bis(2-ethylhexyl) phthalate (2.98%).

These results agree with the study of [36] that found palmitic acid, and linoleic acid and phytol in *S. nigrum*. Phyto diterpene is a diuretic, antibacterial, anticancer, and anti-inflammatory compound. Additionally, phytol was shown to have antibacterial properties against *Staphylococcus aureus* by damaging bacterial cell membranes, which allows potassium ions to escape from the cells [37, 38]. The leaf methanol extract of *S. nigrum* were investigated using gas chromatography mass spectroscopy (GC-MS), *S. nigrum* alkaloid leaf methanol extract revealed the existence of the cyclopentasiloxane-decamethyl, L-proline, dodecanoic acid, 2-pyrrolidinecarboxylic acid-5-oxo, ethyl ester, 1-dodecanamine, 9.12.15-octadecatrienoic acid, Octadecanoic acid, octadecane, 9-octadecenamide, phthalic acid and our results agree with Octadecanoic acid and found phthalic compounds [27].

Table 1. The active compounds in *S. nigrum* using (GC-MS) technology.

No.	Compounds	R.T.	Area%
1	Ethylene glycol, TMS derivative	6.308	0.99
2	4-Cyclopentene-1,3-dione	8.084	0.40
3	Propanoic acid, 2-mercapto-, methyl ester	11.278	0.27
4	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	13.107	0.26
5	(S)-5-Hydroxymethyl-2[5H]-furanone	13.892	0.38
6	5-Hydroxymethylfurfural	14.579	0.77
8	1-Isopropoxy-2,2,3-trimethylaziridine (anti)	17.461	0.35
10	Dodecanoic acid	18.094	0.63
12	3-Methylundecanoic acid	20.976	5.47
13	4,6-Di-O-methyl-.alpha.-d-galactose	21.645	6.01
14	Pentadecanoic acid	22.02	0.25
15	(Z)-4-Decen-1-ol, chlorodifluoroacetate	22.234	0.45
16	cis-7-Hexadecenoic acid	22.859	0.24
17	n-Hexadecanoic acid (Palmitic acid)	23.171	14.64
18	Hexadecanoic acid, ethyl ester	23.34	0.29
19	Heptadecanoic acid	24.001	0.51
20	Heneicosane	24.313	0.25
21	Phytol	24.491	0.41
22	(9E,11E)-Octadecadienoic acid (Linolelaidic acid)	24.83	24.68
23	Octadecanoic acid (Linoleic acid)	25.009	3.98
24	Hexadecane, 1-chloro-	25.214	0.39
26	Octadecane	26.079	0.36
27	1,4-Cyclohexanedimethanamine	26.365	0.58
28	9,12-Octadecadienoic acid (Z,Z)-	26.499	0.32
29	Eicosanoic acid	26.65	1.00
30	Hexanedioic acid, bis(2-ethylhexyl) ester	26.945	0.29
32	Nonadecane	27.703	0.33
33	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	27.855	1.35
34	Bis(2-ethylhexyl) phthalate	28.176	2.98
35	Eicosane	29.229	4.75
36	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	29.666	12.08
37	2-Undecene, 9-methyl-, (E)-	29.755	0.28
39	.alpha.-Tocospiro B	30.451	0.42
40	1-Bromodocosane	30.612	0.96
42	1-Docosanol, methyl ether	32.245	1.56
43	Vitamin E	32.717	0.29
44	Campesterol	33.886	0.55
45	Stigmasterol	34.252	1.47
46	1,4-Bis(trimethylsilyl)benzene	34.582	0.25
47	.gamma.-Sitosterol	35.001	3.64
48	Lupeol	36.188	1.22

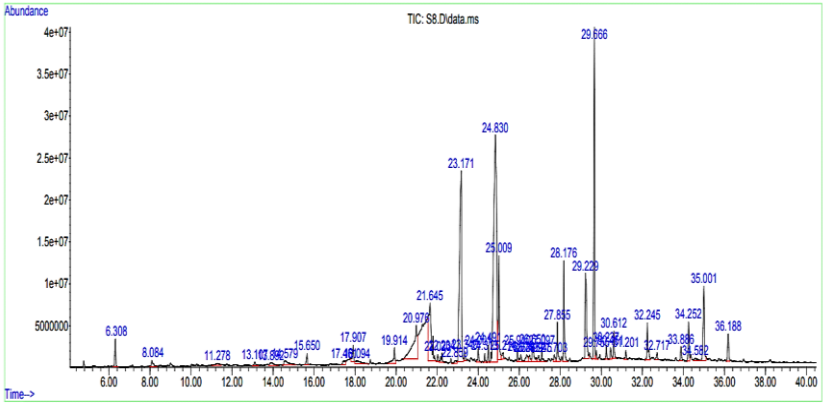


Figure 2. The active compounds of *S. nigrum* using (GC-MS) technology.

3.2. Biogenic synthesis of AgSNNPs.

Figure 3 displays the change of the reaction solution colour from yellowish-green (Figure 3 a) to honey brown (Figure 3 b) after 8 hrs of incubation at 26°C.

(a) (b)

Figure 3. Synthesis of Phyto-biogenic AgSNNPs: (a) The aqueous solution of *S. nigrum*+$AgNO_3$ at time 0, and (b) The aqueous solution of *S. nigrum*+$AgNO_3$ after 8 hrs incubation at 26°C.

The reduction of (Ag^1) into metallic silver (Ag^0), followed by assemblage as small crystals, which ultimately converted to metallic colloidal silver nanoparticles. These results were evidenced by changing the light yellowish-green colour of the aqueous reaction solution of *S. nigrum* leaf extract and silver nitrate to honey brown. These results are in similarity with previous reports [39]. The AgNPs demonstrate a honey-brown colour in aqueous solutions owing to the surface plasmon resonance phenomenon [40].

It has been revealed that plant extracts contain various active compounds, which implicate in the possible synthesis of AgNPs, including, tannins, polysaccharides, ketones, and proteins, flavonoids, terpenoids, vitamins, alkaloids, phenolic acid [26]. As well as antioxidants [41], Since *S. nigrum* possesses high levels of phytochemical compounds as shown in table 1 and Fig 2, thus, we suggest that the construction of AgSNNPs is due to the existence of these agents.

3.3. Characterisation of biosynthesised AgSNNPs.

3.3.1. Optical absorbance analysis of AgSNNPs.

Figure 4 illustrates the absorption spectrum measurements of AgSNNPs with the maximum surface plasmon resonance peak at the range of 429 nm. Our results agree with previous studies, as it has been documented that absorption spectra measurements of biogenic synthesized AgNPs with absorption peaks are concentrated around the range of 420 nm [42]. UV–vis spectrophotometer is the most properly employed strategy for nanoparticle characterization. The existence of the absorbance peak, approximately at 420 nm, reveals the construction of AgNPs owing to surface electrons of nanoparticles that lead to exhibit a unique surface plasmon resonance (SPR). In addition, a position of the absorption peak of AgNPs supplies evidence for the size of AgNPs as the smaller AgNPs exhibit a maximum blue shift in the absorption peak compared to bigger ones. According to the study by Pilaquinga et al. 2019 [43], the average size of AgNPs of 15.3±4.8 nm exhibits an absorption peak of 411.5 nm.

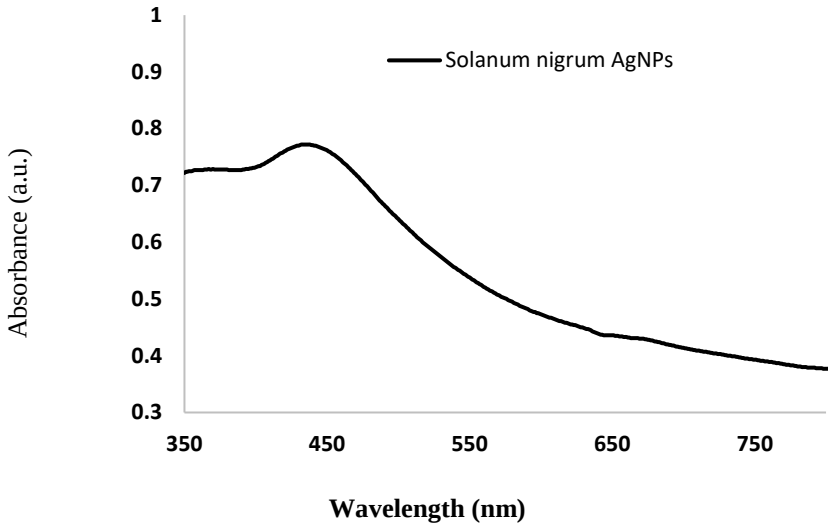


Figure 4. The UV–vis optical absorbance of Phyto-biogenic synthesised AgSNNPs exhibited SPR peak at 420 nm.

3.3.2 Zetasizer analysis of AgSNNPs.

Malvern Instruments Zetasizer Nano ZS (University of Basrah, Iraq), was used to obtain the size distribution of AgSNNPs particles. Figure 5 shows the size distribution of AgSNNPs by number. Zeta-sizer analysis exhibits significant data including the Z-Average (d.nm) of 101.2, the particle size of 19.26, Standard Deviation (6.415), and the sample quality (good). The Polydispersity index (PDI) value (0.226), generated from the distribution outline, indicates that AgSNNPs are sufficiently monodisperse. The data of Standard size distribution analysis is summarized in Table 2. These data are similar to data of other studies [39, 44].

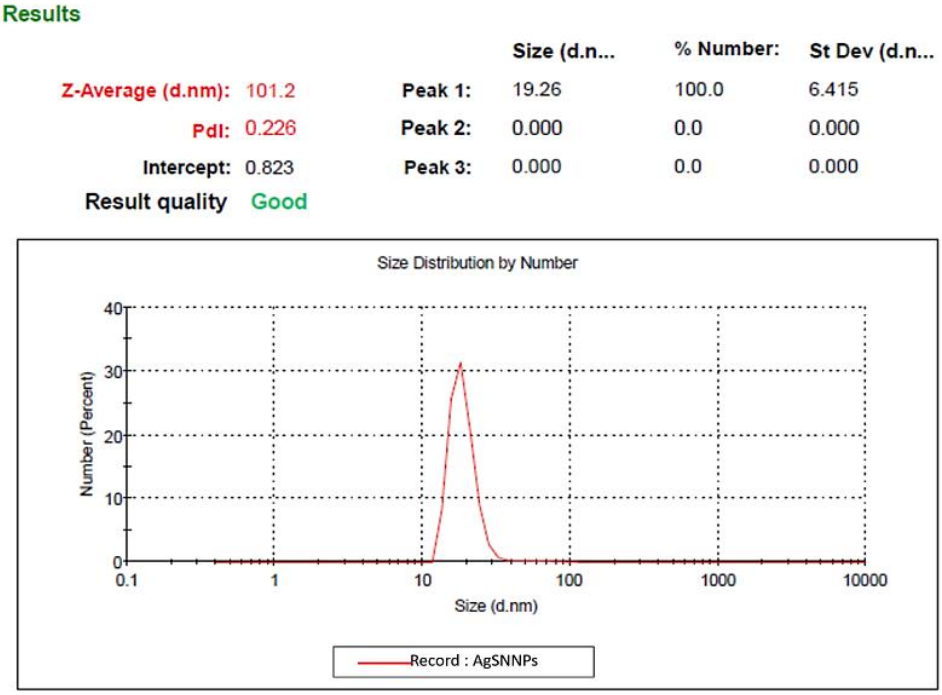


Figure 5. Standard size distribution analysis by a number of AgSNNPs.

Table 2. Size distribution report of Zeta sizer analysis.

Parameter	Value
Z-Average (d.nm)	101.2
PDI	0.226
Size (d.nm)	19.26
% number	100
Standard Deviation	6.415
Intercept	0.823
Result quality	good

3.3.3. Fourier-transform infrared spectroscopy (FTIR) measurements of AgSNNPs.

To identify the active functional groups involved in the synthesis and stabilising the AgSNNPs, FTIR spectra were obtained in a range from 400 to 4000 cm^{-1}. Figure 6 shows the results of the FTIR examination of biogenic synthesised AgSNNPs. It has been observed the presence of a wide extreme absorption peak at a wavelength of 3346.52 cm^{-1} which indicates the presence of a hydroxyl group (OH^-) in phenolic and alcoholic compounds. Moreover, AgSNNPs' FTIR shows three predicted peaks at wavelengths of 2354.08, 1636.93, and 564.86 cm^{-1} [45]. These peaks may result from silver (I) ions exchange with either phenolic or alcoholic active groups that implicate as stabilizers during the AgSNNP fabrication. The analyses were demonstrated by previous studies that discovered the same peak positions from AgNPs. Overall, these findings validated that active components in *Solanum nigrum* extract reduced Ag^+ ions to Ag^0 and covered the surface of synthesised NPs [46, 47].

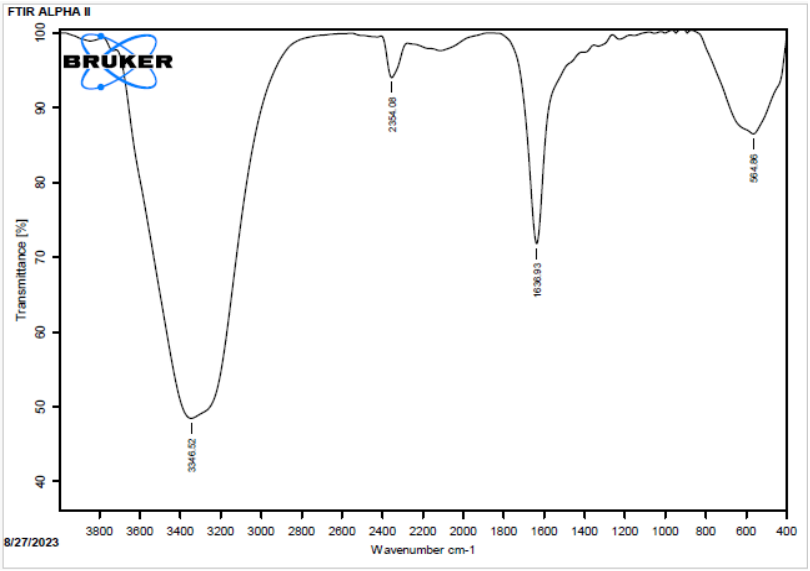


Figure 6. FTIR spectra of AgSNNPs synthesised using *Solanum nigrum* leaf extract.

3.3.4. Field emission electron microscopy (FESEM) measurements of AgSNNPs.

FESEM analysis was conducted to obtain the visual image of synthesised AgNPs using *S. nigrum* extract. Figure 7 represents FESEM image of the Biogenic synthesized AgSNNPs sample. The structural and morphological aspects of AgSNNPs pointed the monodispersed with uniformly spherical shape of AgSNNPs. AgNPs have sizes ranging from 30 to 42 nm with spherical character surfaces. FESEM images revealed that AgSNNPs have homogenous properties without any noteworthy aggregation. Many studies have acquired the same results related to FESEM images [27, 28].

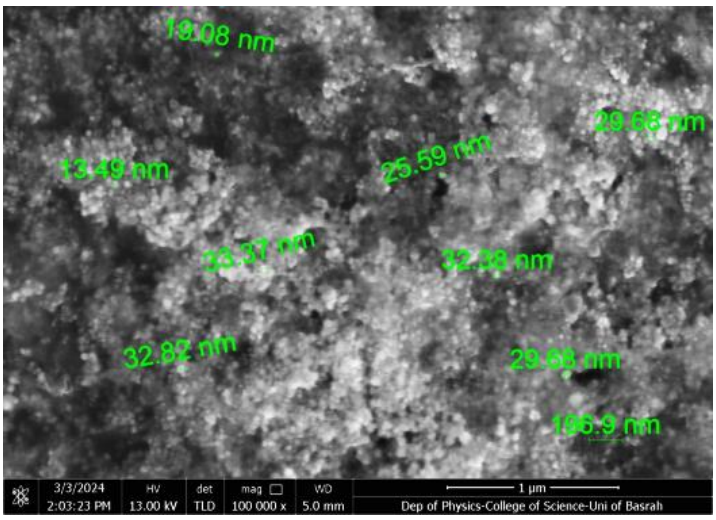


Figure 7. SEM image analysis of AgSNNPs.

3.4. Hemocompatibility evaluation by haemolysis ratio of AgSNNPs.

AgSNNPs synthesized by *S. nigrum* extract showed no haemolytic activity when incubated with red blood cells as shown in Figure 8. In addition, sterilized normal saline that was used as negative control did not show red blood cell haemolysis. However, Triton-X resulted in red blood cells (RBCs) 100% haemolysis after a few minutes of incubation. In general, AgSNNPs synthesized using *S. nigrum* extract were not toxic to RBCs compared to Triton-X. These results suggest, for future investigation, different nanoparticles could be formulated using plant extracts for different biological applications.

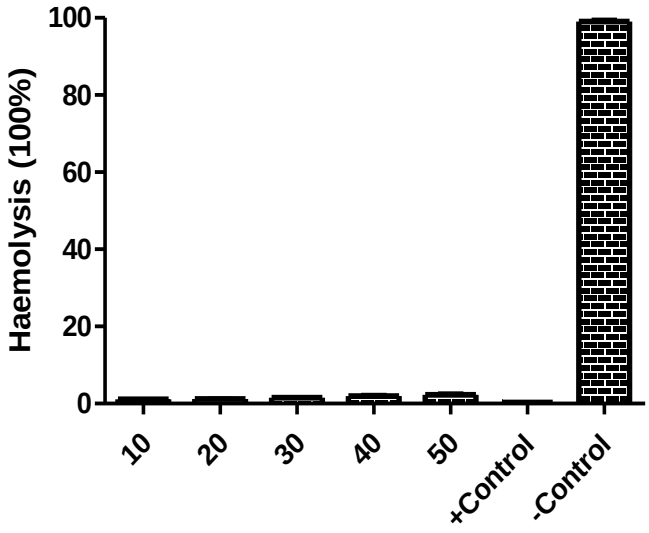


Figure 8. Hemocompatibility evaluation of AgSNNPs.

3.5. Antioxidant evaluation of AgSNNPs.

Solanum nigrum is a medicinal plant, traditionally used to treat pneumonia, painful teeth, stomach pain, tonsillitis, fever, and inflammation, and also as a diuretic antioxidants, anti-tumor and anti-inflammatory [24, 27]. *Solanum nigrum* extract was used in capping of AgSNNPs during the synthesis process. In this investigation, the antioxidant activity of AgNPs was examined using the DPPH scavenging assay [33]. The DPPH has a dark purple colour and absorbs at 540 nm due to its free electron. However, the DPPH colour is decolourized when accepting an electron from an antioxidant compound. The difference in the absorbance of the

DPPH before and after the reaction is due to the antioxidant activity of the antioxidant compound. The antioxidant capability of AgSNNPs demonstrates their effectiveness compared to ascorbic acid as a standard antioxidant agent. In this assay, the DPPH was decolourized using ascorbic acid and AgSNNPs. The results demonstrated that, depending on the concentration utilised, the AgSNNPs had antioxidant activity that was comparable to that of vitamin C. The AgSNNPs showed %50 antioxidant activity at a concentration of 400 µg/ml while ascorbic acid presented %50 antioxidant activity at a concentration of 200-400 µg/ml.

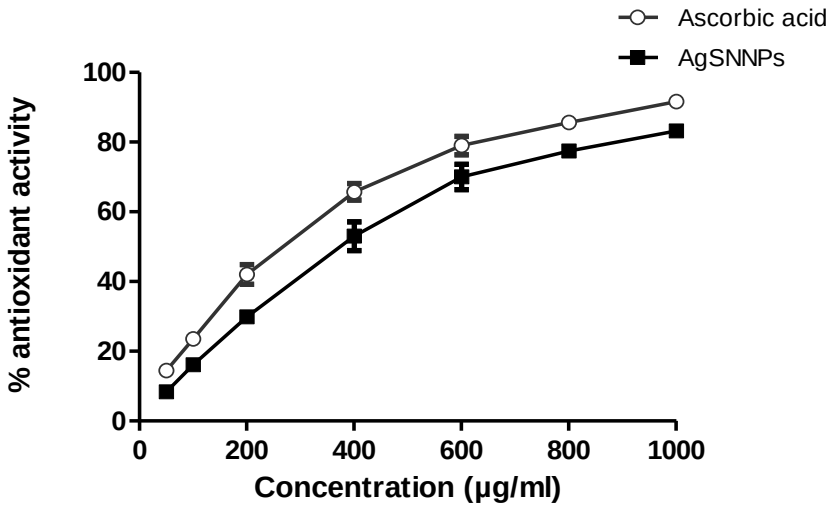
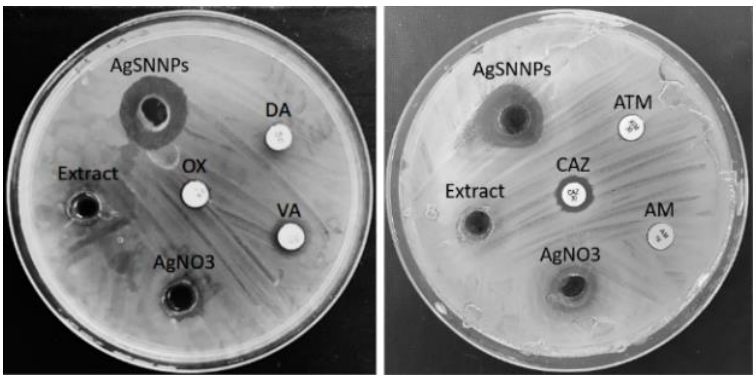


Figure 9. Antioxidant activity of AgSNNPs using DPPH.

3.6. Antibacterial activity of AgSNNPs.

The antibacterial effectiveness of AgSNNPs was evaluated against *S. aureus* and *E. coli* as Gram-positive and Gram-negative bacteria, respectively, using a well diffusion method by measuring the diameters of inhibition zones around wells. The statistical analysis demonstrated significant differences between *S. aureus* and *E. coli* in the measured diameters (Figure 10). As shown in Figure 10 and Table 4, *S. nigrum* extract lacking AgNPs had no antibacterial effects on the two types of bacteria when used with concentration 10 mg\ml. While AgSNNPs (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 6mm against *E. coli*. The inhibition zones diameters of AgSNNPs around wells were with an average of 17.25 ± 1.56 mm and 15.32±1.27 mm for *S. aureus* and *E. coli*, respectively. It is more interested to learn that both isolates were completely resistant to antibiotic discs used in this study (Fig. 9), thus, AgSNNPs held the antibacterial effects compared to the extract, $AgNO_3$ as well as antibiotics. These results agree with a study that revealed that an aqueous extract of *S. nigrum* exhibited antibacterial activity with only a concentration of 100 mg\ml but not with lower concentrations of this extract [48]. A recent study emphasised that the aerial parts of *S. nigrum* extract showed no inhibitory activity against Gram-positive and Gram-negative bacteria compared to antibiotics used in the study [49]. However, our findings in similarity with other studies that indicated different inhibitory activities of AgNPs against pathogenic bacteria particularly the multi-drug resistant bacteria [50, 51].



(a) (b)

Figure 10. The Well diffusion assay of AgSNNPs against (a) *S. aureus* and (b) *E. coli.*

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

Antibacterial agent	Inhibition zone diameters (mm) against:	
	S. aureus	*E. coli*
S. nigrum aqueous extract	6.00±0.00	6.20±0.66
$AgNO_3$ aqueous solution	6.50±0.21	6.54±0.32
Biogenic synthesised AgSNNPs	17.25±1.56	15.32±1.27
Antibiotic of Oxacillin (OX)	0	—
Antibiotic of Vancomycin (VA)	0	—
Antibiotic of Clindamycin (DA)	0	—
Antibiotic of Ceftazidime (CAZ)	—	6.43±0.21
Antibiotic of Ampicillin (AM)	—	0
Antibiotic of Aztreonam (ATM)	—	0

Overall, these findings support the effectiveness of the chosen synthesis technique for AgSNNPs, which involves reducing $AgNO_3$ by *S. nigrum* leaf extract. The results correspond to previous studies in the synthesis AgNPs using different reducing agents which also showed antibacterial efficiency. A study reported that spherical AgNPs exhibit antimicrobial more than other shape such as disk or triangular plate which was depend on the rate of Ag ions released from bacteria and fungi-treated AgNPs [14]. Small AgNPs (10 nm) exhibit more toxicity compared to higher sizes of AgNPs (50 nm), which indicates that Ag ion toxicity depends on the size, type and coating of nanoparticles [52, 54].

4. Conclusions

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

Author Contributions

All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author

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Conflicts of Interest

I hereby confirm that all Figures and Tables in the manuscript are mine. Furthermore, any Figures and images that have been included with permission for re-publication, which is attached to the manuscript. Authors signed on ethical consideration's approval. Ethical Clearance: The project was approved by the local ethical committee at the University of Basra.

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