

Enhancement of the Antibacterial Potential of Biosynthesized silver Nanoparticles by Using Hydrophilic Polymers

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Abstract:

Silver nanoparticles are well recognized for its antibacterial activity, especially against pathogenic bacteria. In the present study, silver nanoparticles (AgNPs) were biologically synthesized by using the aqueous leaves extract of *Datura stramonium* followed by capping with one of the hydrophilic polymers: polyethylene glycol (PEG) and polyvinyl pyrrolidone (PVP). The biosynthesized nanoparticles were characterized by UV–Visible Spectroscopy, X-ray diffraction (XRD) and Transmission Electron Microscope (TEM). Antibacterial activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterococcus* sp. and *Staphylococcus aureus* was determined by a microtitre well method and disc diffusion method. The results revealed that the antibacterial activity of silver nanoparticles is dependent on their concentration and were active against Gram negative and Gram positive bacteria. The antibacterial activity of coated AgNPs was increased in comparison with uncoated.

Keywords: silver nanoparticles, antibacterial activity, *Datura stramonium*, hydrophilic polymers.

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Introduction

Biological synthesis of nanoparticles using nontoxic, eco-friendly approaches is gaining importance due to the growing need to develop clean, nontoxic chemicals, environmentally benign solvents and renewable materials [1]. Among the all noble metal nanoparticles, silver nanoparticles are an arch product from the field of nanotechnology which has gained boundless interests. Silver compounds, silver ions, and silver nanoparticles (AgNPs) have been validated extensively for their antibacterial, antifungal, and antiviral activities [2, 3]. The nanoparticles used for all the aforesaid purposes, the metallic nanoparticles considered as the most promising as they contain remarkable antibacterial properties due to their large surface area to volume ratio, which is of interest for researchers due to the growing microbial resistance against metal ions, antibiotics and the development of resistant strains [4].

Plant leaves, flowers, seeds, tubers, and bark extract mediated biological process for the synthesis of silver nanoparticles has been extensively explored and compared to other bio-inspired processes [5]. Biomolecules present in plant extracts can be used to reduce metal ions to nanoparticles in a single-step green synthesis process. This biological reduction of metal ion to base metal is quite rapid, readily conducted at room temperature and pressure, and easily scaled up [6]. Synthesis mediated by plant extracts is environmentally benign. The reduction and stabilization of silver ions by combination of biomolecules such as proteins, amino acids, enzymes, polysaccharides, alkaloids, tannins, phenolics, saponins, terpinoids and vitamins which are already established in the plant extracts having medicinal values and are environmental benign, yet chemically complex structures [7].

The present research has prompted for further exploration in the use of plant extracts for the biosynthesis of silver nanoparticles from *Datura* leaves extract. *Datura* (family solanaceae) is widely grown all over the world, and used in several traditional medicinal purposes [8]. In contrast to bulk material, the corresponding nanoparticles exhibit high surface energy values, due to their small sizes, and hence often congregate. Therefore, selecting an appropriate capping agent is often a prerequisite for stabilizing nanoparticles [9]. To prevent aggregation of particles, different coating materials, such as carboxylic acids, polymers, polysaccharides, and surfactants, have been exploited to increase stability of the particles [10]. Polyethylene glycols (PEGs) are stable, hydrophilic and have been used as an organic capping agent to stabilize AgNPs and control further growth of the particles [11]. Mekkawy et al., [12] found that PEG coated AgNPs showed higher stability of particles in their water dispersions. Polyvinyl pyrrolidone (PVP) was used as stabilizing agent and prevent the aggregation of silver atoms [13].

Due to the important and critical roles of plants in production of metal nanoparticles, the present research deals with green synthesis of silver nanoparticles (AgNPs) using aqueous leaves extract of *Datura stramonium*. To maintain the nanoparticles dispersed in solutions and prevent their sedimentation, the capping agents tested included polyethylene glycol (PEG), polyvinyl pyrrolidone (PVP) and then the antibacterial activity of uncoated and coated AgNPs against some pathogenic bacteria was studied.

Material and methods:

Biosynthesis of silver nanoparticles:

Aqueous leaves extract of *Datura stramonium* (Jimson weed) was used for the preparation of the silver nanoparticles. Five grams of fresh leaves were boiled in 100 ml distilled water for 5 minutes. After cooling the extract was filtered with Whatman number 1 filter paper. Different concentrations were prepared from the extract 5%. The silver nitrate (AgNO_3) as a source for silver nanoparticles was purchased from Sigma-Aldrich

Chemicals. The extract incubated with silver ion (1mM) for 24 hours. Control without the AgNO₃ was also kept in the same conditions [5].

Characterization of silver nanoparticles:

UV-Visible Spectroscopy analysis

The qualitative testing for confirmation of silver nanoparticles, the optical properties of silver nanoparticles were carried out on Ultraviolet-Visible Spectroscopy (UV-Vis) (Evolution 300- UV-Visible spectrophotometer- England) at a resolution of 1 nm and scanning the spectra between 300-900 nm [14].

Atomic Absorption Spectroscopy (AAS)

The concentration of silver nanoparticles were determined using the atomic absorption spectrometer (Buck model 210 VGP Atomic Absorption Spectrometer-USA) equipped with a silver hollow cathode lamp and air-acetylene flame. Standard silver solutions were also prepared and measured.

Transmission Electron Microscope (TEM)

The morphology and sizes of silver nanoparticles were determined by TEM micrographs using the TECNAI G2 Spirit Twin (MRCE center, Assiut University, Egypt). The sample was prepared by placing a drop of synthesized silver nanoparticles on a negative carbon coated copper grids and dried in air [5].

X-ray diffraction (XRD)

The structure of the biosynthesized silver nanoparticles was checked by X-ray diffraction technique using an X-ray diffractometer (Philips, Amsterdam, Netherlands).

Coating of AgNPs

Effect of coating agent polyethylene glycol (PEG400) and polyvinyl pyrrolidone (PVP) on the AgNPs antibacterial activity was studied. PEG and PVP solutions were prepared by dissolving its powder separately in distilled water. Then, the prepared solution was added directly to the freshly prepared AgNPs suspension to obtain final concentrations of 5 mM of PEG400 and 1 mM PVP coating agents [12]. Stored at 4°C till used.

Antibacterial activity of the biosynthesized silver nanoparticles:

Bacterial strains and growth condition:

The test bacterial strains *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterococcus* sp and *Staphylococcus aureus* were provided from microbiology Lab at the Hospital of Assiut University. Nutrient agar and nutrient broth media were used for growing the tested bacteria.

The antibacterial activity of uncoated and coated AgNPs was monitored with the time (0-10 weeks) against *E. coli*. The MIC, MBC and diameter of inhibition zone for all tested bacteria were determined after 8 weeks from preparation of AgNPs.

Detection of minimum inhibitory concentration (MIC):

To investigate the antibacterial effect of AgNPs on tested bacteria the double fold micro-dilution method on some common pathogens of human was used [12]. Ten concentrations using twofold serial dilution of each of uncoated and coated silver nanoparticles (100 µl in each well) in the 96-well microtitre plate (one plate for each plant extract and one plate for silver nanoparticles) were prepared to begin with 100 ppm for silver nanoparticles. To each concentration 100 µl of 5×10^5 cfu/ ml of the tested were added in a final volume of 200 µl). Pure bacterial inoculum in broth was taken as positive control and nanoparticles and AgNPs without bacterial inoculum was taken as a negative control. The plates were incubated at 30°C for 24 hours and examined for the lowest concentration show no detectable growth (MIC).

Detection of minimum bactericidal concentration (MBC):

After MIC determination of silver nanoparticles, aliquots of 10 µl from all wells in which no visible bacterial growth was observed were seeded in nutrient agar [12]. The plates were then incubated overnight at 30°C. The plates examined for the lowest concentration of antimicrobial agent showed no colony growth of the tested bacteria on the plates (MBC).

Disc diffusion method:

The antibacterial spectrum of the synthesized silver nanoparticles was determined by the disc diffusion method on plates inoculated with the tested bacterial strains [15]. A single colony was inoculated into nutrient broth and over night cultures of the bacterial strains

was prepared in nutrient broth and diluted to 1×10^5 CFU/ml, 200 μ l were stirred well in nutrient agar plates. After solidification, sterile paper discs were placed on the surface of each agar plate and were impregnated with 10 μ l of the silver nanoparticles. The plates were then incubated at 30°C for 24 hours, and then the diameters of inhibition zones were measured to estimate their inhibitory effects. The mean values of inhibition zones were recorded.

Statistical analysis

It was performed by using the SPSS 11 Software.

Results and Discussion

Silver ions and silver salts were used as antibacterial agents [16]. Using plants for the synthesis of silver nanoparticles are ecofriendly, easily available, safe to handle and possess a broad variability of metabolites that may help in reduction [5]. In our study, the leaves extract of *Datura* was used for the green synthesis of silver nanoparticles. The plant extract incubated with silver ion (1mg). Rapid appearance of a yellowish–brown colour in the reaction mixture suggested the formation of colloidal silver nanoparticles (Ali et al. 2015). Visual observation showed that the change in intensity giving brown colour after 24 hours of incubation. This increase in intensity could be due to the formation of more nanoparticles [17].

UV-Visible Spectroscopy analysis

The silver nanoparticles were subjected to spectral analysis by UV–Vis Spectroscopy. The absorption spectra were measured in the range 300–900 nm. AgNPs showed symmetric single-band absorption with peak maximum at 440 nm for 24 h of incubation of cell filtrate with AgNO_3 which steadily increased in intensity as a function of time of reaction without any shift in the peak (Fig. 1). A typical nanoparticles absorption band in the visible region between 350 and 550 nm, Plasmon resonance peak obtained at 420 nm is well-documented for various metal nanoparticles with sizes from 2–100 nm [18]. The

silver nanoparticles band remained around 420 nm indicating that the particles were well dispersed without aggregation [14].

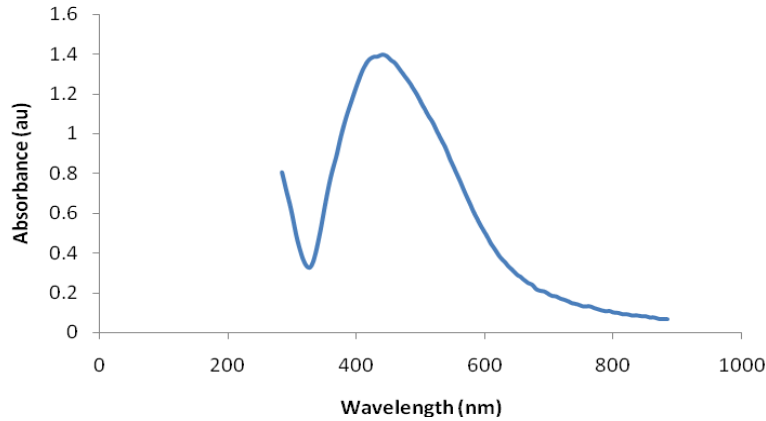


Fig. 1: UV-Visible absorption spectrum of the silver nanoparticles shows a broad absorption band at 440 nm for silver nanoparticles after 24 hours incubation.

Transmission Electron Microscope (TEM)

TEM micrograph of silver nanoparticles revealed that the nanoparticles were mostly spherical in shape and these particles were separated and are not aggregated, indicating stabilization of the nanoparticles and can be used in several applications (Fig. 2). There is a variation in particle sizes from 6-23 nm with an average size approximately 15 nm. Most particles are smaller than 15 nm. This small size range of silver nanoparticles adds to its antibacterial property, since it can easily penetrate bacterial cell membrane and thereafter damage the respiratory chain, affect the DNA, RNA, and division of the cell, and finally lead to cell death [3].

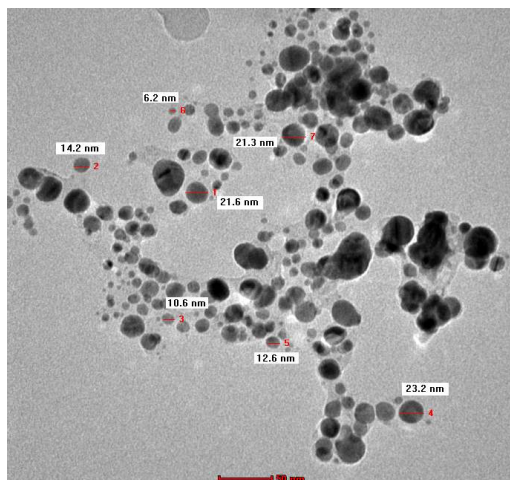


Fig. 2: TEM images of biosynthesized silver nanoparticles using *Datura* leaves extract. The particles are spherical with varying size (AgNPs in the range from 6-23 nm).

X-ray diffraction (XRD)

Due to the crystalline nature of silver nanoparticles, intense X-ray diffraction (XRD) peaks were observed corresponding to the (111), (200), (220), and (311) planes for silver at 2θ angles of 38.02° , 46.12° , 64.6° and 77.26° , respectively (Fig. 3). The XRD analysis confirmed the presence of Ag nanocrystals and no extra diffraction peaks of oxidation or other crystalline phases present, indicating that the pure silver nanoparticles are crystalline in nature. The obtained data was matched with the Joint Committee on Powder Diffraction Standards (JCPDS files no. 03-0921). A comparison of the obtained XRD spectrum with the standard confirmed that the silver particles formed in our experiment were in the form of nanocrystals. The XRD spectrum in the present study agrees with Bragg's reflection of silver nanocrystals, similar reported in other literature [19].

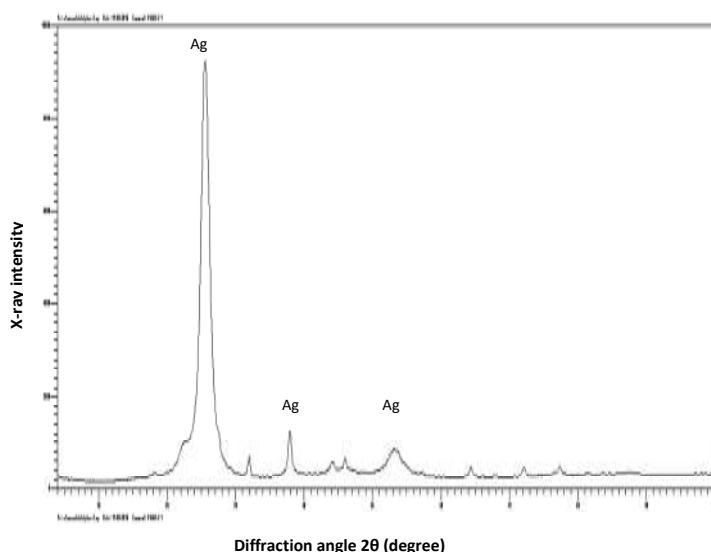


Fig. 3: The XRD images of biosynthesized AgNPs using *Datura* leaves extract.

Antibacterial activity of biosynthesized silver nanoparticles:

The preliminary study of the antibacterial activity of biosynthesized silver nanoparticles which synthesised by using plant extract, it was noticed that the antibacterial activity of AgNPs was decreased with the time. In this study the antibacterial activity of the uncoated or coated AgNPs was moitored with the time (0-10 weeks) by disc diffusion method against *E. coli*. The coated AgNPs exhibited more dispersion and antibacterial activity in comparison with uncoated AgNPs (data not shown). The MIC, MBC and diameter of inhibition zone of bacterial strains tested were detemined after 8 weeks from the preparation of all AgNPs.

The biosynthesized AgNPs showed high inhibitory effect against *E.coli* (Table 1). This may be due to the action of AgNPs on the DNA and inactivation of cellular protein [20]. Moreover, it was suggested that silver ions bind to functional proteins, resulting in protein denaturation [21, 22]. Also, highly reactive metal oxide nanoparticles, lead to significant increase in permeability of bacterial membrane, resulting in the incapability of

bacterial cells of properly regulating transport through plasma membrane and causing cell death [22].

The antibacterial activity of uncoated AgNPs and coated AgNPs with capping agents was studied against five bacterial species (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterococcus sp.* and *Staphylococcus aureus*) Table 1. Coated AgNPs with PEG and PVP showed 3.12 µg/mL MIC against *E. coli* and *S. aureus* and 6.25 µg/mL MIC against *K. pneumoniae*, *P. aruginosa* and *Enterococcus sp.* On other hand uncoated AgNPs showed lower MIC against all bacterial strains tested.

Using uncoated AgNPs showed MBC value for all bacterial strains tested at concentration 50 µg/mL and their MIC was 25 µg/mL except *Staphylococcus* showed the same MBC and MIC. In respect to AgNPs coated with PVP against *E. coli* and *Pseudomonas* MBC (6.26 and 12.5) was higher than MIC (3.12 and 6.25), respectively. There are various values of MIC reported for AgNPs against *Staphylococcus* or *Streptococcus* strains in the literature, most probably due to differences in the size, physicochemical properties and methods of synthesis [23]. The average of MIC value 4.86 µg/mL was reported for 25 nm AgNPs against *Streptococcus* mutants [24]. Sherma et al., [25] found that MIC and MBC for *E. coli* were 0.62 and 1.25 respectively. In addition Espinosa-Cristóbal et al., [26] found much higher MIC against *Streptococcus*: 101.98, 145.64, and 320.63 µg/mL for AgNPs with the size of 8.4, 16.1, and 98 nm, respectively. For *Staphylococcus* and *E. coli* MIC and MBC values were in the range 0.9-3.75 and 3.75-4.5 µg/mL, respectively [12].

Table 1: Susceptibility of bacterial strains to silver nanoparticles (AgNPs).

Bacterial strain	MIC (µg/mL)			MBC (µg/mL)		
	Uncoated AgNPs	Coated AgNPs		Uncoated AgNPs	Coated AgNPs	
		PVP	PEG		PVP	PEG

<i>E. coli</i>	25	3.1	3.12	50	6.2	6.25
<i>Klebsiella</i>	25	6.25	6.2	50	12.5	12.5
<i>Pseudomonas</i>	25	6.25	6.2	50	6.25	12.5
<i>Enterococcus</i>	25	6.25	6.2	50	6.25	6.25
<i>Staph. Aureus</i>	25	3.12	3.12	25	6.25	6.25

MIC is Minimum Inhibition Concentration, MBC is Minimum Bacterial Concentration,

PVP is polyvinyl pyrrolidone, PEG is polyethylene glycol.

The antimicrobial activity exhibited by silver nanoparticles is shown in the graph of inhibition zone of five bacteria as a function of increasing concentration of nanoparticles. In general PEG or PVP coated AgNPs showed more inhibitory effect against all bacterial strains tested than uncoated AgNPs (Fig. 4). However all bacterial strains except *P. aeruginosa* was highly inhibited by AgNPs coated with PEG while, AgNPs coated with PVP showed the highest effect against *P. aeruginosa*. Similar to the current result, Amato et al., [27] demonstrated that the coated AgNPs exhibited higher antibacterial activity than the uncoated AgNPs. It is worthy to mention that, AgNPs capped with beta-cyclodextrin enhanced the inhibitory effect of nanoparticles against *E. coli*, *P. aeruginosa* and *Staph. aureus* [2]. PEG-coated AgNPs showed greater stability as indicated by a decreased sedimentation rate of particles in their water dispersions and MIC and MBC values for *Staph. aureus* and *E. coli* were in the range of 0.93–7.5 and 3.75–15 µg/mL, respectively [12].

The results also indicate that an increase in the concentration of silver nanoparticles reduced the survival of bacteria. In this respect, Pal et al., [28] reported that, the AgNPs undergo shape-dependent interaction with Gram-negative *E. coli* and were being used as bactericidal agents, and also agrees with Kim et al., [29] investigated the inhibitory effect of AgNPs on microorganisms.

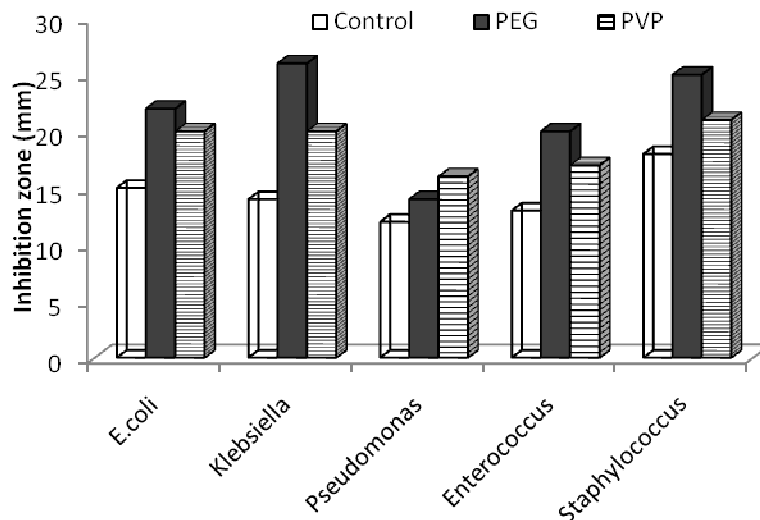


Fig. 4: The mean value of inhibition zone caused by different AgNPs. Control is uncoated AgNPs, PEG is AgNPs coated with polyethylene glycol and PVP is AgNPs coated with polyvinyl pyrrolidone.

Although, the exact mechanisms by which silver nanoparticles act as antibacterial agent are not fully understood, there are several theories. Silver nanoparticles can anchor onto bacterial cell wall and with subsequent penetration, perforate the cell membrane (pitting of cell membrane) ultimately leading to cell death [30]. The dissipation of the proton motive force of the membrane in *E. coli* occurs when nanomoles concentration of silver nanoparticles is given [31]. Earlier studies with electron spin resonance spectroscopy revealed that free radicals are produced by silver nanoparticles in contact with bacteria, which damage cell membrane by making it porous, ultimately leading to cell death [32].

Conclusions

In the present study, the silver nanoparticles AgNPs were biosynthesized by using leaves extract of *Datura stramonium*. TEM and AAS were used to study the morphology, size and concentration of AgNPs. The silver nanoparticles exhibited distinct antimicrobial property on AgNPs coated with capping agents observed to be the most stable and to display the highest antibacterial activity. The hydrophilic polymers PEG and PVP maintained the stability and enhanced the antibacterial activity of AgNPs. The described

microtitre plate assay could be used as a rapid and sensitive method for detection of bacterial growth inhibition. This eco-friendly, cost-effective extracellular biosynthesis of silver nanoparticles with potent antimicrobial activities from the plant extracts has the potential to be utilized on a large scale for widespread industrial or medical application.

Competing interests

The authors declare that they have no competing interest.

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