Antimicrobial, antioxidant and antitumor activities of silver nanoparticles synthesized by *Allium cepa* extract: A green approach

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**Abstract** An economic and efficient method for the synthesis of silver nanoparticles (AgNPs) was performed using onion (*Allium cepa*) extract as reducing and capping agent. UV–vis spectroscopy confirmed the formation of silver nanoparticles by observing the typical surface plasmon resonance peak at 420 nm. Transmission electron microscopy studies revealed that AgNPs were spherical in shape with a size range of 10–23 nm. AgNPs were further demonstrated by the characteristic peaks observed in the XRD image. The possible functional groups of AgNPs were identified by FTIR analysis. AgNPs exhibited potential antimicrobial activity against all the microbial strains tested. Antioxidant activity of AgNPs revealed that they can be used as potential radical scavenger against deleterious damages caused by the free radicals. Additionally, AgNPs had antitumor activities against human breast (MCF-7), hepatocellular (HepG-2) and colon (HCT-116) carcinoma cell lines in a dose-dependent manner with IC50 of 1.6, 2.3 and 2.2 μg/ml, respectively. The overall results indicate promising baseline information for the potential uses of AgNPs in the treatment of infectious diseases and tumors.

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**1. Introduction**

The field of nanotechnology is considered as one of the most important and active areas of research. Its fundamental building blocks, nanoparticles, are distinguished by some exclusive physicochemical properties such as optical, electronic, catalytic and magnetic ones. These unique properties of nanoparticles could be attributed to high surface area in comparison to volume ratio, which potentially results in high reactivity [6].

Silver nanoparticles (AgNPs) have been reported to be applied in agricultural, medical and environmental fields [11]. Additionally, this type of nanoparticles is the best choice for potential applications in the field of living organisms and biological systems.

The increased usage of nanoparticles especially for medical purposes created a new challenge of synthesizing them by untraditional methods to overcome the undeniable disadvantages of other physical and chemical methods [21]. Plant
mediated nanoparticles synthesis (green method) is preferred as it is clean, cost effective, nontoxic and safe for human therapeutic use [4].

Onion (*Allium cepa*) is a widely cultivated plant all over the world. It is rich in carbohydrates, proteins, sodium, potassium and phosphorus. It has been reported to have numerous important properties such as antimicrobial, antioxidant, antiparasitic and anti-inflammatory activities [7]. Recently, it is found that onion can be used as a potential candidate for synthesis of silver nanoparticles.

The aim of the present study was to evaluate the use of onion extract as a reducing agent for silver nanoparticle formation. The synthesized AgNPs were then characterized by UV–vis spectroscopy, TEM, EDAX, XRD, and FTIR. Further, some biological activities including antimicrobial, antioxidant and antitumor properties of the produced silver nanoparticles were studied.

2. Materials and methods

2.1. Materials

Onion (*Allium cepa*), used as a reducing agent for synthesis of silver nanoparticles, was obtained from the local market. All tested microorganisms were obtained from Fermentation Biotechnology and Applied Microbiology Center, Al-Azhar University, Cairo, Egypt.

2.2. Synthesis of silver nanoparticles

Onion extract was prepared by finely crushed 50 g of fresh onion followed by filtration. The extract has to be free from debris. The extract was then mixed with 100 ml deionized water, boiled for 8 min. One ml of onion extract was mixed with 10 ml of 0.1 mM AgNO₃ (analytical grade, Sigma Aldrich) with constant stirring at 60–65 °C. Silver nanoparticles formation was observed by the color change as described by Lekshmi et al. [8]. AgNPs were collected by centrifugation at 15,000 rpm for 20 min. Dispersion of AgNPs in de-ionized water followed by centrifugation were repeated three times to confirm purification. Onion extract and AgNPs were stored as lyophilized powder.

2.3. Characterization

The synthesized silver nanoparticles (AgNPs) were characterized by UV–vis spectrum, TEM, EDAX, XRD and FTIR analysis. All these analyses were carried out at Nanotechnology Characterization Center (NCC), Agriculture Research Center (ARC), Giza.

2.3.1. UV–vis spectroscopy

Synthesis of AgNPs was assured by measuring the UV–vis spectrum of the reaction mixture. The absorption spectrum was recorded over the range of 300–800 nm using UV–vis spectrophotometer (Cary 5000, Varian, Australia).

2.3.2. Transmission electron microscopy (TEM) and EDX analysis

Morphology and size of the AgNPs were investigated by TEM images using Tecnai G20, Super twin, double tilt, 200 kv instrument (FEI, Netherland). A small amount of the sample was dropped on a carbon coated copper grid and drying under lamp to form a thin film of the sample. The interaction of the electrons transmitted through the specimen resulted in the formation of an image which is then magnified and focused onto an imaging device. EDX (Energy Dispersive X-ray) analysis of the synthesized AgNPs was carried out using the same instrument to investigate the elemental composition of the sample.

2.3.3. X-ray diffraction (XRD)

The formation and quality of compounds were checked by X-ray diffraction (XRD) spectrum. A coated film of AgNPs was formed on a glass plate and XRD pattern was investigated using INEL X-ray diffractometer (Co-κα1 radiation (λ = 1.78 Å) in the range of 10° to 80°at a scan rate of 0.05°/min with the time constant of 2 s).

2.3.4. Fourier-transform infrared spectroscopy

FT-IR measurements were used to identify the possible biomolecules associated with AgNPs formation. The infrared spectrum of the sample was measured with KBr disk in the wavelength range of 4000–400 cm⁻¹ using Perkin Elmer FTIR spectrophotometer (Jasco 6100, Japan).

2.4. Biological activities of the AgNPs

2.4.1. Antimicrobial assay and MIC determination

Antimicrobial activity of the synthesized AgNPs was evaluated using the agar well diffusion method [15]. The agar plates had been seeded with tested microorganisms. Wells of 6 mm diameter was punched using a sterile cork borer and filled with 250 μl of the samples. Onion extract was tested as control. Each experiment was repeated three times and the mean diameters of the inhibition zones were recorded in millimeters. The minimal inhibitory concentration (MIC) was investigated using the micro-dilution broth method. Briefly, the tested bacterial isolates were grown in nutrient broth and the turbidity of cultures was adjusted to 0.5 according to McFarland standard. Serial 2-fold dilutions of AgNP extracts ranging from 0.078 to 10 mg/ml were prepared in nutrient broth. Equal volume of each diluted extract was added to bacterial cultures and then incubated at 37 °C for 24 h. The lowest concentration caused inhibition of the visible growth is recorded as the MIC value of the sample.

2.4.2. Antioxidant assays

The synthesized AgNPs were evaluated for antioxidant activity using 3 methods as follows. The average of the results for each experiment was calculated. Onion extract was tested as control and L-Ascorbic acid (AA) was used as reference.

2.4.2.1. Scavenging of DPPH radicals

The efficiency of AgNPs for scavenging DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical was assessed using the method of Chang et al. [3]. A serial of dilutions of AgNPs were prepared in methanol (200, 100, 50, 25 and 12.5 μl/ml). To each dilution, equal volume of DPPH (0.135 mM in methanol) was added, vortexed thoroughly and left in the dark at room temperature for 30 min. Control was prepared as described above without extract. The absorbance of the samples and that of control were estimated at
517 nm. The ability of AgNPs to scavenge the DPPH radical was recorded as a percentage of inhibition as follows:

\[
\text{% Inhibition} = \left( \frac{\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}}}{\text{OD}_{\text{Control}}} \right) \times 100
\]

2.4.2.2. Total antioxidant assay. The total antioxidant activity of AgNPs was evaluated following the method described by Prieto et al. [17]. An aliquot of 0.3 ml of each diluted extract prepared as described above was mixed with 3 ml of reagent solution (4 mM ammonium molybdate, 28 mM sodium phosphate and 0.6 M sulfuric acid), incubated at 95 °C for 90 min. After cooling the tubes, the absorbance of the solution was measured at 695 nm and the antioxidant activity was calculated as% following this equation:

\[
\text{Total antioxidant} (\%) = \left( \frac{\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}}}{\text{OD}_{\text{Control}}} \right) \times 100
\]

2.4.2.3. Reducing power. Fe\(^{3+}\) reducing power of AgNPs was determined following by the method described by Oyaizu [13]. AgNPs extract at various concentrations (0.75 ml) was mixed with equal volumes of phosphate buffer (0.2 M, pH 6.6) and K\(_2\)Fe(CN)\(_6\) (1%, w/v), incubated at 50 °C for 20 min. Trichloroacetic acid (TCA) solution (10%) was added to stop the reaction and then centrifuged at 15,000 g for 10 min. After adding FeCl\(_3\) solution (0.1%, w/v) to the diluted supernatant for 10 min, the absorbance was measured at 700 nm. As the absorbance of the reaction mixture increased, reducing power of the extract increased.

2.4.3. Antitumor assay

The cytotoxicity potential of the synthesized AgNPs was studied against human breast (MCF-7), hepatocellular (HepG-2) and colon (HCT-116) carcinoma cell lines obtained from the American Type Culture Collection (ATCC, UAS). The cell viability was measured using MTT assay which is based on the reduction of the tetrazolium salt by actively growing cells [18]. Dulbecco’s modified Eagle’s medium (DMEM) amended with 1% L-glutamine, HEPES buffer, 50 μg/ml gentamycin and 10% heat-inactivated fetal bovine serum was used for cultivation of the cells in 24-well plate for 48 h at 37 °C in 5% CO\(_2\). The formed monolayer of cells was exposed to various concentrations of AgNPs (50, 25, 12.5, 6.25, 3.125, and 1.56 μg/ml) for 48 h. The percentage of cell viability was calculated as follows:

\[
\text{Cell viability (\%)} = \left( \frac{\text{OD}_{\text{experimental group}}}{\text{OD}_{\text{control group}}} \right) \times 100.
\]

IC\(_{50}\) extract concentration causing 50% inhibition was calculated from the graph plotting percentage of cell viability against extract concentration. The lower the IC\(_{50}\) value indicates high antitumor capacity.

2.5. Statistical analysis

All experiments were carried out in triplicate, and the results were presented as mean ± standard deviation. The experimental data were analyzed using SPSS. Statistical significance was accepted at a level of p < 0.05.

3. Results and discussion

In this study, silver nanoparticle (AgNP) synthesis by reducing Ag\(^{+}\) ions present in the aqueous solution of silver nitrate with the help of onion (Allium cepa) extract was investigated. The color change from colorless to dark brown indicated the formation of AgNPs [19]. Change in the state of a matter from the molecular scale to nano scale is accompanied with color change due to excitation of surface plasmon vibrations in nanoparticles [18].

3.1. UV–vis spectroscopy

UV–visible spectroscopy is an important technique used to confirm the formation of metal nanoparticles in an aqueous solution. As shown in Fig. 1 UV–vis absorption spectrum of the produced AgNPs showed an absorbance peak at 420 nm due to excitation of surface plasmon vibrations in nanoparticles. One of the most important features in the optical absorbance spectra of metal nanoparticles is surface plasmon band, which is due to collective electron oscillation around the surface mode of the particles [16,20]. The presence of a single surface plasmon resonance band in the absorption spectra of the produced AgNPs gives an indication to their spherical shape [5].

3.2. TEM and EDAX analysis

The image of TEM showed that the produced AgNPs are spherical in shape with a size ranging from 10 to 23 nm (Fig. 2). Energy Dispersive Analysis of X-ray (EDAX) gives qualitative as well as quantitative status of elements that may be involved in the formation of AgNPs. Data illustrated in Fig. 3 showed the peak in silver region at 3KeV (54.39% in mass) which is typical for the absorption of metallic silver nanocrystalline due to surface plasmon resonance. Further, weaker signals from C and O atoms are also recorded. Thus the transmission electron microscopy gave descriptions of shape, size and structure of the biosynthesized AgNPs in details.

3.3. XRD spectrum

The synthesized AgNPs were further confirmed by the characteristic peaks observed in XRD image as shown in Fig. 4. AgNPs were in the form of nanocrystals as evidenced by the peaks at 20 values of 38.10°, 44.28°, 64.42° and 77.37°. The intensity of 100% was found at 20 value with 38.10°. Other observed peaks suggested that the crystallization of bio–organic phase occurs on the surface of the silver nanoparticles [22].

3.4. FTIR analysis

FTIR measurement was carried out to identify the possible bio–molecules responsible for capping and efficient stabilization of AgNPs synthesized by onion extract. Active functional groups in the synthesized AgNPs are confirmed by the presence of some absorption bands in the spectrum as shown in Fig. 5. The band at 3424 cm\(^{-1}\) corresponds to O–H stretching.
H-bonded alcohols and phenols. The peak at 2921 cm$^{-1}$ corresponds to O–H stretch carboxylic acids. The assignment at 1625 cm$^{-1}$ corresponds to N–H bend primary amines. The peak at 1387 cm$^{-1}$ corresponds to C–N stretching of aromatic amine group and the bands observed at 1061 and 971 cm$^{-1}$ corresponds to C–N stretching alcohols, carboxylic acids, ethers and esters. Therefore, the synthesized AgNPs were surrounded by proteins and metabolites such as terpenoids having functional groups of aldehydes, ketones, alcohols and carboxylic acids [1]. The carbonyl group from the amino acid residues has the stronger ability to bind metal indicating that the proteins could prevent the molecules to be in clusters and stabilize AgNPs in the aqueous medium [22].

3.5. Antimicrobial activity

The antimicrobial activity of the synthesized AgNPs was evaluated against several pathogenic gram positive and gram
negative bacteria as well as a fungus (*Candida albicans*). As recorded in Table 1, AgNPs showed a pronounced antimicrobial activity against all the tested microorganisms as compared to onion extract (control). *Klebsiella pneumonia*, *Proteus vulgaris* and *Serratia marcescens* showed resistance to onion extract, however they were inhibited by AgNPs formed by the same extract. The highest activity of AgNPs was found against *Pseudomonas aeruginosa* ATCC 10145 and *Bacillus subtilis* NCTC10400 reached 29 and 27 mm, respectively which is higher than that previously reported by Manikandan et al. [9].

To compare the sensitivity of the microbial strains to the extracts, MIC test was done and the data are presented in Table 2. There were substantial differences between the MICs of AgNPs and onion extract. AgNPs had the most effective antimicrobial activity. Since nanoparticles have large surface area and small size, they can bind and interact to the cell more effectively than the large particles. The attachment of silver nanoparticles to the surface of the cell membrane not only caused disturbance of permeability and respiration processes but also caused inhibition of the enzyme functions and inactivation of DNA replication because the high affinity of silver toward sulfur and phosphorus, respectively [10].

### 3.6. Antioxidant activity

Oxidation is an essential biological process in many living organisms for the production of energy; however, the
uncontrolled production of oxygen derived free radicals. Reactive oxygen species (ROS) caused damage of complex cellular molecules such as carbohydrates, proteins, lipids and DNA [24]. This led to the appearance of many health problems like cancer, cardiovascular diseases, liver diseases, renal failure, inflammatory problems, and aging in general [23].

Antioxidants are agents that, in one way or another, restrict the deleterious effects of these oxidant reactions. These restrictions can involve scavenging free radicals or preventing radical formation and therefore can enhance the immune defense and lower the possibility of diseases occurrence [14]. The search for new antioxidants is of great importance to avoid the side effects and diseases caused by synthetic ones.

In this study, antioxidant activity of the synthesized AgNPs was investigated using 3 different assays because evaluation of antioxidant activity cannot be carried out accurately by single universal method. DPPH scavenging capacity test is the best choice for the measurement of antioxidant activity because of its stability (in radical form), simplicity, and fast assay. Antioxidants, on interaction with DPPH, either transfer electrons or hydrogen atoms to DPPH, thus neutralizing the free radical character and decreasing its absorbance [12]. The phosphomolybdenum method of total antioxidant capacity test based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and formation of a green phosphate/Mo(V) complex. Further, the potassium ferricyanide reduction method may serve as a significant indicator of potential antioxidant activity. The results presented in Fig. 6–8 showed that AgNPs have higher antioxidant activity as compared to onion extract or ascorbic acid and their activity increased with concentrations.

Table 1 Antimicrobial activity of onion extract biosynthesized silver nanoparticles (AgNPs) against some pathogenic microorganisms.

| Microorganism                          | Inhibition zone diameter (mm) |
|----------------------------------------|-------------------------------|
|                                        | Onion extract | AgNPs            |
| Bacillus subtilis ATCC 6633             | 15 ± 0.15      | 25 ± 1.22        |
| Bacillus subtilis NCTC 10400            | 10 ± 0.11      | 27 ± 0.54        |
| Bacillus cereus ATCC14579               | 16 ± 0.10      | 22 ± 1.66        |
| Bacillus licheniformis ABRII6           | 11 ± 0.12      | 25 ± 1.20        |
| Bacillus sp. 2BSG-PDA-16                | 10 ± 0.02      | 19 ± 0.24        |
| Bacillus sp. DV2-37                    | 11 ± 1.10      | 22 ± 0.55        |
| Staphylococcus aureus NCTC 7447        | 15 ± 0.09      | 23 ± 1.30        |
| Streptococcus mutans ATCC 3654         | 15 ± 0.03      | 20 ± 1.6         |
| Escherichia coli NCTC 10418            | 11 ± 0.00      | 24 ± 0.39        |
| Klebsiella pneumoniae ATCC 10031        | –               | 11 ± 0.56        |
| Salmonella typhimurium NCIMB 9331      | 14 ± 0.02      | 20 ± 0.88        |
| Pseudomonas aeruginosa ATCC 10145      | 15 ± 0.07      | 29 ± 1.60        |
| Proteus vulgaris ATCC27973              | –               | 15 ± 1.0         |
| Serratia marcescens ATCC 25179         | –               | 12 ± 1.4         |
| Cida albicans ATCC 70014               | 5 ± 0.02       | 14 ± 0.2         |

Values are means ± SD of 3 separate experiments; –, no inhibition zone.

Fig. 5 FT-IR spectrum of the biosynthesized silver nanoparticles (AgNPs).
Table 2  Minimum inhibitory concentration (MIC) of onion extract biosynthesized silver nanoparticles (AgNPs) against some pathogenic microorganisms.

| Microorganism                  | MIC (mg/ml) | Onion extract | AgNPs |
|-------------------------------|-------------|---------------|-------|
| *Bacillus subtilis* ATCC 6633 | 1.25        | 0.312         |       |
| *Bacillus subtilis* NCTC 10400| 5           | 0.156         |       |
| *Bacillus cereus* ATCC14579   | 2.5         | 0.625         |       |
| *Bacillus licheniformis* ABRII6| 5           | 0.312         |       |
| *Bacillus* sp. 2BSG-PDA-16    | 2.5         | 0.625         |       |
| *Bacillus* sp. DV2-37         | 5           | 0.312         |       |
| *Staphylococcus aureus* NCTC 7447| 2.5        | 0.312         |       |
| *Streptococcus mutans* ATCC 3654| 1.25       | 0.312         |       |
| *Escherichia coli* NCTC 10418 | 1.25        | 0.312         |       |
| *Klebsiella pneumonia* ATCC 10031| –         | 2.5           |       |
| *Salmonella typhimurium* NCTM 9351| 1.25       | 0.312         |       |
| *Pseudomonas aeruginosa* ATCC 10145| 1.25      | 0.156         |       |
| *Proteus vulgaris* ATCC 27973 | –           | 0.625         |       |
| *Serratia marcescens* ATCC 25179| –          | 0.625         |       |
| *Cida albicans* ATCC 70014    | 10          | 0.625         |       |

– extract not tested.

Fig. 6  DPPH free radical scavenging activity of onion extract (OE), biosynthesized silver nanoparticles (AgNPs) Ascorbic Acid (AA) at different concentrations.

Fig. 7  Total antioxidant capacity of onion extract (OE), biosynthesized silver nanoparticles (AgNPs) Ascorbic Acid (AA) at different concentrations.
3.7. Antitumor activity

Cancer is a multi-step, dangerous and widely distributed disease. Oxidative damage as well as various chemical, physical, environmental and genetic factors can directly or indirectly induce its synthesis. There is always a need for new and effective agents to control this disease. Various reports revealed that silver nanoparticles have important anti-angiogenic properties. Compounds possessing anti-angiogenic properties may have antitumor activity as they have the ability to block the activity of abnormally expressed signaling protein [2].

In the present study, antitumor activity of the synthesized AgNPs at different concentration (50, 25, 12.5, 6.25, 3.125 and 1.56 µg) was studied In vitro against human breast cancer cell lines. The synthesized AgNPs were found to be effective against human breast cancer cell lines with IC50 values ranging from 1.6 to 3.13 µg.

Table 3  Inhibition concentrations (IC50) for cytotoxic activity of onion extract biosynthesized silver nanoparticles (AgNPs) on human breast (MCF-7), hepatocellular (HepG-2), colon (HCT-116) carcinoma cells.

| Human carcinoma cells | Onion extract (µg) | AgNPs (µg) | Doxorubicin (µg) |
|-----------------------|-------------------|------------|-----------------|
| MCF-7                 | 11.2              | 1.6        | 0.426           |
| HepG-2                | 4.3               | 2.3        | 1.2             |
| HCT-116               | 5.0               | 2.2        | 0.469           |
(MCF-7), hepatocellular (HepG-2) and colon (HCT-116) carcinoma cell lines and compared with the standard Doxorubicin. AgNPs showed higher cytotoxic potential against all cell lines in a dose-dependent manner than the onion extract alone, as shown in Figs. 9–11. The results cited in Table 3 showed that MCF-7 cells proliferation was significantly inhibited by AgNPs with an IC50 value of 1.6 μg/ml. Furthermore, AgNPs exhibited moderate cytotoxic activities against HepG-2 and HCT-116 cell lines (IC50 of 2.3 and 2.2 μg/ml, respectively).

The results illustrated in this study suggested that the onion mediated synthesized silver nano-particles possess great antimicrobial, antioxidant and antitumor potentials. This green method of silver nanoparticle formation opens a new window for the treatment of various infectious diseases and tumors.

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