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The Antioxidant Activity and Cytotoxic Effects of *Amaranthus cruentus*-Biosynthesized Silver Nanoparticles Toward MCF-7 Breast Cancer Cell Line

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Abstract

Introduction: Silver nanoparticles (AgNPs) have grabbed special attention owing to their exclusive structural features. Green synthesis (i.e., plant-mediated) of AgNPs is an efficient and cost-effective method with widespread clinical applications. Therefore, the present study aimed to synthesize AgNPs based on green synthesis method employing the seed extracts of *Amaranthus cruentus* and to investigate the antioxidant and cytotoxic activities of the biosynthesized AgNPs.

Methods: The Ag-NPs were biologically synthesized using the *A. cruentus* extract which served as a reducing agent. Then, the synthesized Ag-NPs were visualized by transmission electron microscopy. Next, the antioxidant activity of the synthesized Ag-NPs was evaluated by DPPH and ABTS methods. Finally, the cytotoxicity of AgNPs was investigated against MCF-7 breast cancer cell line using MTT assay.

Results: The mean diameter of the synthesized Ag-NPs ranged from 20 to 40 nm. In addition, the IC_{50} of free radical scavenging activity of the Ag-NPs were obtained as 500 µg/mL (DPPH) and 400 µg/mL (ABTS). Further, the AgNPs showed time and dose-dependent cytotoxicity against MCF-7 cells. Eventually, at the 24-hour exposition to the 80 µg/mL dose of AgNPs, the viability of cancerous cells was 19% plunging to 2.03% and 1.9% after 48 hours and 72 hours, respectively.

Conclusion: In general, plant extracts can serve as facile and eco-friendly alternatives to hazardous methods for synthesizing the metal nanoparticles. Therefore, the *A. cruentus* biosynthesized Ag-NPs can be utilized in medicine for various purposes due to their low toxicity and appropriate antioxidant activity.

Keywords: Silver nanoparticles, Biological synthesis, *Amaranthus cruentus,* Transmission electron microscopy

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Introduction

Silver nanoparticles (AgNPs) have many technological, clinical, and industrial applications[1-4] and are effectively applied in medicine as antibacterial, antifungal, and antitumor agents.[5-8] Among many nanoparticles (NPs), AgNPs have grabbed specific attention due to their specific properties and stability.[9,10] These NPs can be produced by several physicochemical approaches[11] among which the physical methods are expensive and highly energy-consuming.[12] On the other hand, chemical methods including sol-gel techniques require costly metal salts and exploit harmful solvents such as sodium borohydride, hydrazine, and the like which can be potentially toxic toward the living cells.[13,14] Therefore, developing feasible and environmentally safe strategies are desirable for producing the NPs.[15-18] In this regard, plant extracts are employed for safe biosynthesis of AgNPs (i.e., green synthesis).[19,20]

Plants contain a wide range of biologically active constituents such as phenolic acids, anthocyanins, flavonoids, and several other biocomponents.[21,22] *Amaranthus cruentus* which belongs to the Amaranthaceae plant family is used in medicine mainly due to its antimicrobial properties.[23] In addition, AgNPs are considered as the most characterized materials which are utilized in many fields of nanoscience and nanotechnology.[24] AgNPs, along with many unique biomedical properties exhibit

potent antioxidant and cytotoxic activities thus, these NPs are used as helpful elements in surgical procedures, wound healing processes, and food and water decontamination approaches.[25]

In the present study, AgNPs were synthesized based on green synthesis method using the seed extracts of *A. cruentus*. Further, the antioxidant and cytotoxic activities of the biosynthesized AgNPs were studied as well.

Materials and Methods

Amaranthus cruentus Seed Extraction

First, an experienced taxonomist characterized the plant identity and then, the seed extract of *A. cruentus* was used to synthesize AgNPs. To prepare the seed extract, 50 g of seed particles were sliced into small pieces and submerged in 500 mL of distilled water. Next, the admixed was refluxed for four hours. Finally, the extract was refrigerated at 4°C for further use.

Biosynthesis and Microscopic Characterization of AgNPs

The AgNPs were synthesized by adding 10 mL of *A. Cruentus* seed extract to 90 mL of silver nitrate aqueous solution with a concentration of 1 mM. The solution was prepared in a 100 mL round-bottom flask equipped with a shaker. Then, the reaction mixture was shaken for ~24 hours at room temperature. Next, the solution was centrifuged for 30 minutes at 9000 rpm and then washed several times using the deionized water. Eventually, the synthesized AgNPs were visualized by transmission electron microscopy (TEM, Zeiss EM 10 C- 100 kV, Germany).

Antioxidant Assays

The antioxidant activity of the synthesized AgNPs was assayed employing 2, 2-Azinobis-3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) and DPPH methods.

ABTS Test

First, ABTS radical cation (i.e., $ABTS^{+}$) was produced by adding ABTS solution (7 mM) to ammonium-persulfate (2.45 mM). This solution was then incubated in the dark at 25°C for 16 hours. ABTS solution was first diluted with PBS (pH 7.4) and the absorbance was read at 734 nm in order to measure the antioxidant activity of the synthesized NPs. Next, diluted ABTS (1 mL) was admixed with 10 mL of either AgNPs or butylated hydroxy anisole (BHA). Then, the radical scavenging capacity was determined based on the reduction in the absorbance of the test solution using a BHA standard curve and the following equation.

$$\text{Radical scavenging percentage (ABTS)} = \frac{A_c - A_s}{A_c} \times 100$$

In this equation, *Ac* and *As* represent the absorbance of control and sample, respectively.

DPPH Test

In brief, 23 mg/mL of DPPH solution was dissolved in ethanol and then the absorbance of the solution was measured at 517 nm. In this test, the DPPH, as a free radical, was reduced to diphenylpicrylhydrazine following exposition to antioxidants. The radical scavenging capacity was calculated using the formula below.

$$\text{Radical scavenging percentage (DPPH)} = \frac{A_c - A_s}{A_c} \times 100$$

where, *Ac* and *As* denote the absorbance of control and sample, respectively. The control solution was prepared by mixing ethanol and DPPH radical solution.

AgNPs Cytotoxicity Against MCF-7 Cell Line

MCF-7 breast cancer cell line was purchased from the Pasteur Institute of Iran (Tehran, Iran). The cells (~1×104 per well) were cultured in 96 well plates containing DMEM medium and incubated at 37°C for 48 hours. Then, the MCF-7 cells were treated with different concentrations of green synthesized AgNPs (10–100 µg/mL) for 24 hours and 48 hours. Cytotoxicity in each concentration was measured using the MTT method.

Statistical Analysis

Statistical analysis was conducted by the SPSS software, version 22. The results were expressed as means ± standard deviation (SD). The between-group comparisons were conducted using non-parametric statistics. A *P* value of <0.05 was considered statistically significant.

Results

Figure 1 illustrates the transmission electron microscopy image of the synthesized Ag-NPs which has a spherical shape with a mean particle diameter of about 25 nm.

Based on the ABTS method shown in Figure 2, free radicals inhibition ratio ranges from 27% to 66% at 127

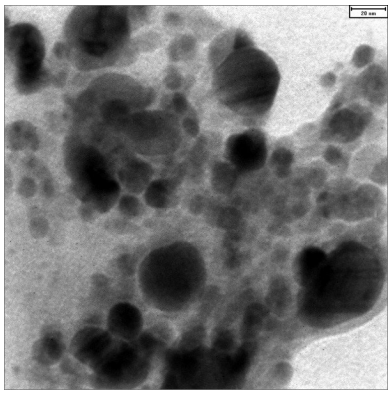


Figure 1. Transmission Electron Microscopy Image of Biosynthesized Silver Nanoparticles

to 1000 µg/mL concentrations of AgNPs, respectively, delivering as IC_{50} of 400 µg/mL.

As displayed in Figure 3, the biosynthesized AgNPs show 22% to 58% free radical scavenging capacity at 125 to 1000 µg/mL concentrations, respectively using DPPH method, demonstrating an IC_{50} of 500 µg/mL.

Figure 4 depicts the viability of MCF-7 breast cancer cells which were treated with the biosynthesized AgNP. The in vitro cytotoxicity of the AgNPs is assessed against the breast cancer cell line (MCF-7) utilizing the MTT assay. The AgNPs demonstrate time and dose-dependent cytotoxicity against the MCF-7 cells. At 24 hours exposition to the NPs, the viability of cancerous cells is 19% plunging to 2.03% and 1.9% after 48 hours and 72 hours expositions to 80 µg/mL AgNPs, respectively.

Discussion

Nanomedicine is regarded as a rapidly growing field in medicine. In fact, using the NPs has emerged promising horizons in cancer therapy. The present cancer therapeutics (i.e., chemotherapy and radiotherapy) are largely restricted due to the associated drug toxicities, side effects, as well as low efficacy and the specificity of the drug on cancerous cells, and finally, the development of chemoresistance.[26,27] Furthermore, plant-synthesized NPs provide suitable therapeutic substitutes for the treatment of cancer and thus can be used in both passive and active targeted therapies against cancerous cells and tissues. Moreover, NPs can be exploited as drug delivery mediators.[28] Nevertheless, NPs are mainly employed in association with other cancer therapeutics since their use as the sole platforms in cancer is yet challenging.[29] In the current study, AgNPs were synthesized using *A. cruentus* (i.e., green biosynthesis). NPs were synthesized by this approach deliver high specificity, biocompatibility, safety, and efficacy in parallel to lower toxicity and therefore, can be considered as appropriate anti-cancer agents.

The cytotoxicity results showed that the biosynthesized

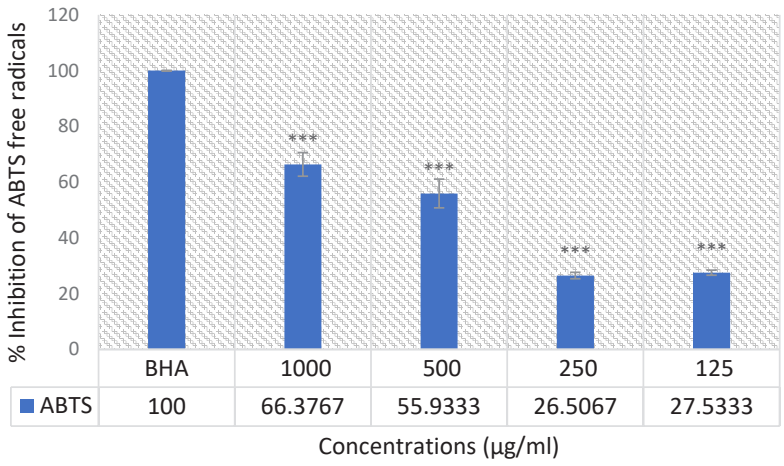


Figure 2. The Inhibition Ratio of Free Radicals at Different Concentrations of AgNPs Using the ABTS method. *Note*. *** indicates a significant difference compared to the control group.

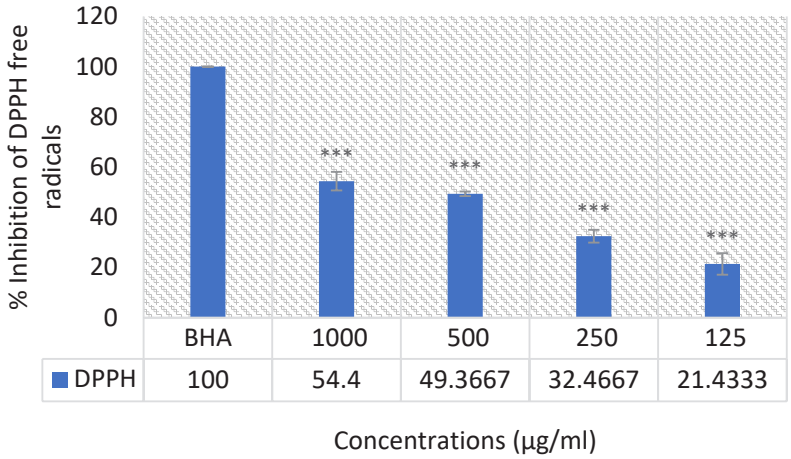
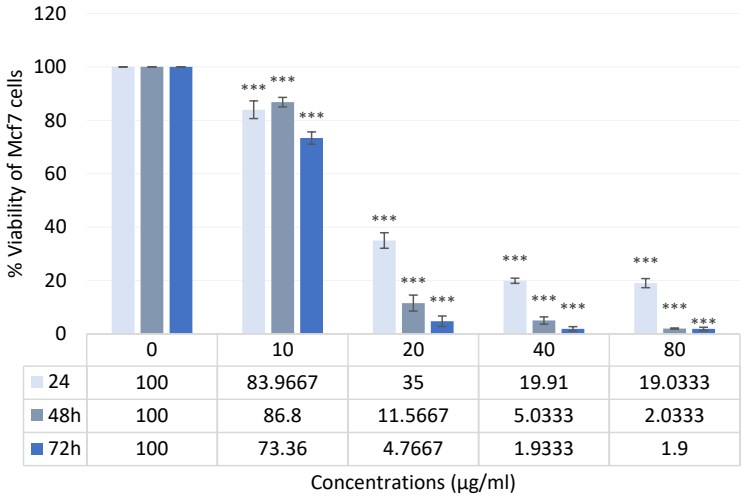


Figure 3. The Ratio of Free Radical Inhibition at Different Concentrations of AgNPs using DPPH Method. *Note*. *** displays a significant difference in comparison with the control group.



	0	10	20	40	80
24	100	83.9667	35	19.91	19.0333
48h	100	86.8	11.5667	5.0333	2.0333
72h	100	73.36	4.7667	1.9333	1.9

Figure 4. The viability of MCF-7 Breast Cancer Cells Treated With Biosynthesized Silver Nanoparticle After 24, 48, and 72 Hours. *Note*. *** denotes a significant difference when compared to the control group.

AgNPs had dose-dependence toxicity against MCF7 cancerous cell line with dramatically decreased cell viability at higher concentrations and longer exposition to AgNPs. Regarding their excellent safety profile, the green-synthesized NPs can be as appropriate agents for cancer therapy. In fact, antioxidants such as polyphenols, flavonoids, anthocyanin, and saccharides available in medicinal plants can augment the antioxidant activities of the green-synthesized NPs.[30]

In the present research, the antioxidant activity of the synthesized AgNPs was measured utilizing DPPH and ABTS methods. DPPH (2, 2-diphenyl-1- picrylhydrazyl) is a free and stable radical.[31] The delocalization of the DPPH molecule creates a purple color with a maximum absorbance around 517 nm. Additionally, the reduction of DPPH by hydrogen donors leads to the changes in color from deep violet to light yellow, which corresponds to the reducing capacity of the target compound.[32] The $ABTS^{+}$ with the peak absorbance at 743 nm delivers a bluish-green color which is raised by the loss of an electron by the nitrogen atom of ABTS (2,2-azino-bis3-ethylbenzthiazoline-6-sulphonic acid).[33] ABTS can be oxidized by potassium persulphate[34] generating ABTS cation radicals ($ABTS^{\bullet+}$). Based on the results of the present study, AgNPs demonstrated high antioxidant activities. The IC_{50} values obtained for the synthesized AgNPs were 500 µg/mL and 400 µg/mL in DPPH and ABTS methods, respectively. These IC_{50} values indicated the concentration of AgNPs required to rummage 50% of DPPH or $ABTS^{\bullet+}$ free radicals. Plant extracts themselves contain diverse molecules with distinct antioxidant activities.[35] Therefore, widespread antioxidant properties of the green synthesized NPs (e.g., AgNPs produced in the present study) are justifiable as well. Nevertheless, the silver ions (Ag^{+}) present in the structure of AgNPs are noted as the potential toxic agents, regardless of their method of synthesis.[36] Overall, further investigation is required to evaluate *in vivo* and *in vitro* effects of the green synthesized AgNPs.

Conclusion

In general, metal NPs can be biologically synthesized using the plant extracts as a facile and eco-friendly alternative to hazardous physicochemical methods. The bioreduction of silver ions is one of the most common methods for the synthesis of the colloidal form of AgNPs. In the present study, Ag^{+} ions were reduced using *A. Cruentus* extract in order to synthesize AgNPs which delivered a safe and easy procedure. In addition, the biosynthesized AgNPs showed prominent antioxidative properties by scavenging free radicals. Furthermore, the obtained AgNPs demonstrated significant cytotoxic effects on MCF-7 breast cancer cell line. Ultimately, based on the results, the synthesized AgNPs using *A. Cruentus* extract are recommended as potential therapeutic agents for cancer treatment.

Ethical Approval

Not applicable.

Competing Interests

The authors declare no competing interests.

Acknowledgments

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