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Green Synthesis of Silver Nanoparticles Based on *Scutellaria* sp. Stem Extract and Its Antitumor Activity

Anticancer acivity of green synthesized $AgNO_3$-NPs using *Scutellaria* extract

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Abstract— **Biosynthesis of nanoparticles using various plant materials is classified as a green technology because this production method does not employ toxic chemicals. The main purpose of this research was to identify the potential for *Scutellaria* sp. stems to synthesize silver nanoparticles ($AgNO_3$-NPs) by a simple green method and to evaluate its efficacy. *Scutellaria* sp. stems were used to synthesize silver nanoparticles by the bio-reduction of silver nitrate ($AgNO_3$). The UV–Vis, Field emission scanning electron microscope (FESEM), X-ray diffraction analysis (XRD), and Fourier transform infrared spectroscopy (FT-IR) techniques were used to characterize these particles. The surface plasmon resonances were measured using UV–Vis spectroscopy. The crystallization, structural, and morphological configurations were investigated by FE-SEM and XRD, respectively. Functional groups were identified using FT-IR. Nanoparticles were evaluated for their antitumor potential against MDA-MB-231 cell line to determine the enhanced toxicity responses in cancerous cells. The silver nanoparticles were formed in 1.5 hours by sonication at room temperature. In this case, a dark brown color was developed. The successful formation of silver nanoparticles was confirmed by UV–Vis, FESEM, FT-IR and XRD analysis. The characteristic peaks of the UV-Vis spectrum and XRD confirmed the synthesis of $AgNO_3$-NPs. The biosynthesized $AgNO_3$-NPs showed potential anticancer activity against MDA-MB-231 cell line. The results clearly suggest effective anticancer activity of green biosynthesized $AgNO_3$-NPs that can be developed for nano-pharmacological relevance in biomedical applications.**

Keywords-Silver nanoparticles; Scutellaria* sp.*; Green synthesis; Anti-tumor activity

1. Introduction

In the last decade, green synthesis of metal nanoparticles has been exponentially increasing because of its various applications, such as electronics, catalysis, chemistry, energy, and medicine [1]. In particular, silver nanoparticles ($AgNO_3$-NPs) have many important applications in several fields, such as high sensitivity biomolecular detection and diagnostics [2], antimicrobials [3], catalysis [4], optics [5], biomedicine [6] and medicine [7]. Conventional methods for manufacturing $AgNO_3$-NPs are expensive, environmentally toxic, and/or hazardous. Thus, green synthesis methods for $AgNO_3$-NPs are highly required. Extracts from various plants, such as *Rhus coriaria* [8], *S. baicalensis* [9] and *Thymbra spicata* [10] are promising substances for a green and facile synthesis of $AgNO_3$-NPs.

Scutellaria sp. is a perennial herb distributed in Tabriz, Iran, that grows on steep west and southwest-facing slopes 1400-1800 m above sea level with an annual rainfall of 230-2700 mm [11]. *Scutellaria* sp. has been used for many years as a medicinal herb in Iran and has been applied in the treatment of hemorrhage, inflammation and infections. The flavonoids and terpenoids found in roots are credited for these biological efficacies [11, 12]. Flavones such as baicalein, wogonin and chrysin are the major bioactive compounds extracted from the roots and shoots of *Scutellaria* sp. [12]. These flavones have been reported to have various biological activities, including anti-inflammatory, anticancer, antibacterial and antioxidant, antiviral, anticonvulsant, hepatoprotection, and neuroprotective effects [13, 14]. In the present study, we report that an aqueous stem extract of *Scutellaria* sp., placed in a concentrated aqueous solution of $AgNO_3$, resulted in reduction of the silver ions to form $AgNO_3$-NPs. These green-synthesized $AgNO_3$-NPs of *Scutellaria* sp. were examined by UV–Vis spectroscopy, FESEM, XRD, and FTIR. $AgNO_3$-NPs were characterized and analyzed for their anticancer activity against MDA-MB-231 cell line.

2. Materials and methods

2.1. Collection of Plant Material

Scutellaria sp. fresh stems were collected from Tabriz Province, Iran. The taxonomic identity of the plant was confirmed by the Biology Department of Tabriz University, Iran [11].

2.2. Biosynthesis of $AgNO_3$ nanoparticles From Scutellaria sp. Aqueous Extract

About 5 g of dried stems were crushed and heated with 100 ml of deionized water. The mixture was heated at 90°C for 30 min. Then the mixture was cooled and filtered through Whatman No.1 filter paper. Afterwards it was centrifuged at 4000 rpm and freshly used. 1, 2.5 and 5 mL of the filtrate was treated with aqueous 0.5, 1 and 2 mM $AgNO_3$ solution in an Erlenmeyer flask and sonicated for 30 min (three times) and then incubated at room temperature for 24 h. 2 mM AgNO3 as the final concentration was added to the 5 mL stem extract as the optimum condition, resulting in a dark brown solution indicating the formation of $AgNO_3$-NPs [8].

2.3. Characterization of Biosynthesized $AgNO_3$-NPs

The absorbance of the resulting solutions was measured with a spectrophotometer at a range 300–700 nm at a resolution of 1 nm. Presence of a peak in the range of 425 nm indicated synthesis of $AgNO_3$-NPs. To observe $AgNO_3$-NPs morphology, FESEM analysis was performed by Gold coating of $AgNO_3$-NPs.

Crystallographic Structure (XRD) of green synthesized $AgNO_3$-NPs was achieved in the range of 2θ from 20° to 80° using Cu Kα radiation of wavelength 1.5406 Å. Moreover, FT-IR spectroscopy of $AgNO_3$-NPs were carried out using KBr pellet technique wave number ranging 400–4000 cm^{-1}.

2.4. MTT Assay on Human Cancer Cell Lines

MTT assay was performed to test the cytotoxic effect of synthesized $AgNO_3$-NPs on breast cancer (MDA-MB-231) cell line according to the reported procedure [14]. Briefly, cells were cultured in DMEM supplemented with 10% FBS and 1% antibiotics (streptomycin and penicillin). Cells at a density of ~100,000 were seeded in 96-well flat-bottom plates and incubated for 24 h at 37 °C in the incubator with 5% CO_2 supply. After 24 h, the test material (in triplicate) was added at different concentrations (500, 250, 125, 60, 30 µg/mL) to the wells containing the MDA-MB-231 and HFF2 cell lines. Untreated cells and the blank samples (cells with DMSO used as solvent) were used as controls. The plate was incubated again at 37 °C with 5% CO_2 in the incubator for 24 hrs. Cell viability was then determined as follows: 10 µL MTT reagent (5mg/mL) was added to each treated sample, blank, and untreated cells and incubated at 37 °C for 3 h. After that, 100 µL of the solubilization solution was added to the wells, and incubation was done in the dark at room temperature for 2–4 h. Finally, the absorbance of the samples was measured at 570 nm in the plate reader.

3. Results and Discussion

3.1. Biosynthesis and characterization of AgNO3-NPs

According to the results, the reduction process occurring to aqueous $AgNO_3$ was confirmed by the formation of deep brown nanoparticles (Figure. 1a). The peak as the characteristic of the surface plasmon resonance (SPR) of $AgNO_3$-NPs was observed at 425 nm (Figure. 1b). 2 mM $AgNO_3$ as the final concentration was added to the 5% *Scutellaria* sp. stem extract and sonicated for 30 min (three times) as the optimum factor in the process of synthesizing $AgNO_3$-NPs.

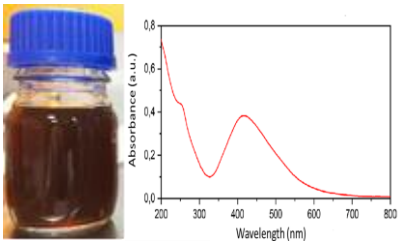

Figure 1. Dark brown color (a) and UV visible spectrum (b) of synthesized $AgNO_3$-NPs

The morphological characterization of silver nanoparticles was examined by FE-SEM, which is the preferred method to directly measure morphology, size distribution, grain size, and nanoparticle size [15]. As shown in Figure 2a, the results of FE-SEM analysis revealed that the biosynthesized $AgNO_3$-NPs were spherical in shape with sizes ranging from ~ 20 to 40 nm.

The crystal structure of the biosynthesized nanoparticles was evaluated by XRD technique (16). The XRD pattern for $AgNO_3$-NPs confirmed the presence of [111], [200], [220], and [311] lattice planes of Bragg's reflections (Figure 2b).

FT-IR measurements were carried out to identify the possible biomolecules responsible for the reduction of the Ag^+ ions and capping of the bio-reduced silver nanoparticles synthesized by *Scutellaria* sp. stem extract. Figure 3 represents the FT-IR spectrum of nanoparticles.

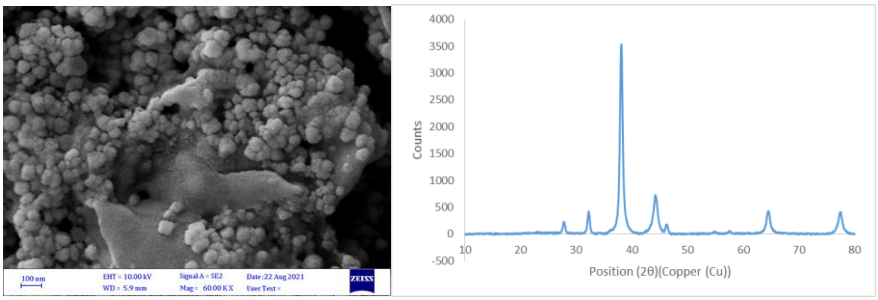

Figure 2. FESEM (a) and XRD (b) images of biosynthesized $AgNO_3$-NPs.

FT-IR peaks showed the different functional groups. Synthesized silver nanoparticles showed almost similar absorption peaks in regions already related to the presence of polyphenols capped by $AgNO_3$-NPs [17]. The FT-IR spectra had major vibration modes at 731, 1104, 1270, 1459, 1633, 1722, 2065, 2866, 2928 and 3422 (Figure 3). All these spectra represented different functional groups. FT-IR studies suggested the presence of various groups of secondary metabolites.

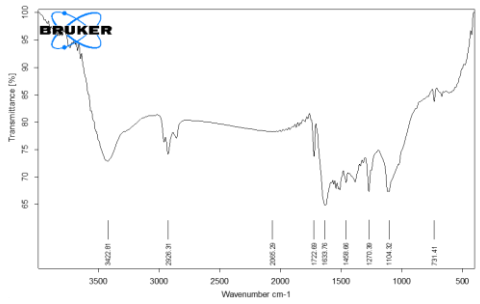

Figure 3. Fourier transform infrared (FT-IR) spectrum of biosynthesized silver nanoparticles using *Scutellaria* sp. stem.

3.2. Anticancer assay

In this study, the treated cells with several concentrations of the $AgNO_3$-NPs were examined by MTT test for 24 h regarding the cytotoxicity properties on normal (HFF2) and breast adenocarcinoma (MDA-MB-231) cell lines (Figure 4). In the case of MDA-MB-231 adenocarcinoma cells, the viability of them reduced dose-dependently in the presence of $AgNO_3$-NPs. The best result of cytotoxicity property of $AgNO_3$-NPs and a significant decrease in cell viability was seen at 250 µg/mL.

The IC_{50} of $AgNO_3$-NPs against MDA-MB-231 cell line was 54.7 µg/mL. The cytotoxic responses of the $AgNO_3$-NPs, suggesting that biosynthesized $AgNO_3$-NPs could contribute in search of alternative chemotherapeutic agent.

Our results showed more than 70% of cell death at 250 µg/ml of $AgNO_3$-NPs. The cytotoxic effects induced by $AgNO_3$-NPs could be due to the plant components attached to the $AgNO_3$-NPs [18].

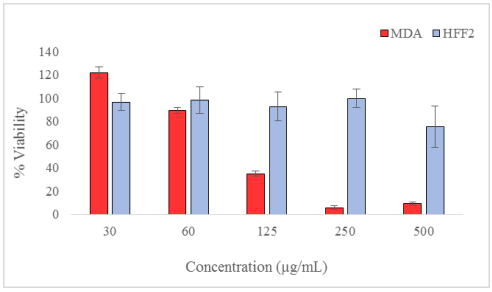

Figure 4. MTT assay results. Cytotoxic effects of dispersed $AgNO_3$-NPs in the *Scutellaria* sp. stem extract and $AgNO_3$-NPs on cancer cell line (MDA-MB-231) and normal cell line (HFF2). Data are calculated as mean ± SD of three experiments.

Conclusion:

The $AgNO_3$-NPs have been successfully synthesized using *Scutellaria* sp. stem extract as base source and stabilizing agent. The synthesized $AgNO_3$-NPs were spherical in shape and were characterized using UV-Vis, XRD, FESEM and FTIR techniques. The biosynthesized nanoparticles had effective anti-breast cancer effects against breast adenocarcinoma (MDA-MB-231) cell line without any cytotoxicity activity against normal cell line i.e., HFF2. These obtained results showed that $AgNO_3$-NPs synthesized in *Scutellaria* sp. extract are potentially useful for cancer treatments.

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