

Biosynthesis of silver nanoparticles using extract of olive leaf: synthesis and in vitro cytotoxic effect on MCF-7 cells

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Abstract A green synthetic approach using olive leaf extract was optimized for preparing silver nanoparticles (AgNPs). Parameters affecting the formation of nanoparticles such as temperature, exposure time to extract, pH, concentration of silver nitrate, and extract (ratio of plant sample to extraction solvent) were investigated and optimized. Optimum conditions for the synthesis of silver nanoparticles are as follows: Ag⁺ concentration 1 mM, extract concentration, 8 % w/v; pH = 7, time, 4 h and temperature, 45 °C. Synthesized silver nanoparticles were characterized using UV–Visible absorption spectroscopy, Fourier-transform infrared spectroscopy, X-ray diffraction, dynamic light scattering (DLS), and scanning electron microscopy (SEM). SEM and DLS analysis showed that the synthesized AgNPs were predominantly spherical in shape with an average size of 90 nm. The cytotoxicity activities of the synthesized AgNPs and olive leaves extract containing AgNPs against human breast cancer cell (MCF-7) were investigated and the inhibitory concentration (IC₅₀) was found to be 50 and 0.024 µg/mL at 24 h incubation, respectively. This eco-friendly and cost-effective synthesis method can be potentially used for large-scale production of silver nanoparticles.

Keywords Silver nanoparticles · Olive leaf extract · Anticancer · MCF-7

Background

Green chemistry is the design of chemical products and processes that reduce or eliminate the use or generate hazardous substances for human health and environment. Therefore, Green chemistry protects the environment, not by cleaning up, but by introducing new chemical processes that do not pollute the environment.

Nowadays, development of green synthesis of metallic nanoparticles and their applications is one of the most important areas of research. For synthesizing of silver nanoparticles (AgNPs), several methods such as chemical synthesis [1], electrochemical [2], radiation [3, 4], photochemical [5], and biological synthesis [6–9] have been used. In comparison with other methods, biological methods for synthesis of AgNPs are environmental friendly. Due to this reason, these methods have been preferred. Among the biological methods, the use of plant extracts for the synthesis of AgNPs is not only simple and cost-effective but also the synthesized particles are stable. Recently, a rapid, energy-efficient, green, and economically scalable room temperature method for synthesis of stable AgNPs using the tannic acid (a polyphenolic compound derived from plant extract) was developed [10].

The antibacterial activities of AgNPs are dependent on the size, shape, and stabilizing agents of the nanoparticles [11–13]. The antibacterial activities increased with size reduction of AgNPs. Aggregation of nanoparticles causes reduction in antibacterial activities of AgNPs. Therefore, combination of nanoparticles with stabilizer agents prevents the aggregation and lead to maintain antibacterial activities of AgNPs.

Despite the widespread use of AgNPs in medical fields, relatively few in vitro studies have been accomplished to determine the cytotoxicity effects of AgNPs

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on different cell lines [14–18]. In all these studies, the AgNPs were removed from extract (plant extract) after the synthesis and their anticancer activities were studied. However, there is no study on anticancer activities of AgNPs in the presence of plant extracts. This is the first report on the anticancer activities of olive leaves extract containing AgNPs.

Phenolic compounds are important minor components that can be commonly found in many plants [19]. However, these compounds have antioxidant activity and health benefits [20, 21]. Olive oil, fruit, and leaves contain high levels of polyphenol compounds [22, 23] that can be contributed to the reduction of silver ions.

In this work, synthesis of AgNPs using aqueous olive leaves extract was investigated and optimized. The cytotoxicity effects of extract containing AgNPs on cell viability were studied using human breast cancer cells (MCF-7).

Methods

Chemicals

Silver nitrate, sodium hydroxide, Ammonia solution (25 %) and orthophosphoric acid were purchased from Merck Chemical Co. (Darmstadt, Germany). All solutions were prepared with distilled water.

Instrumentation

UV–Vis spectra were recorded using a UV–Vis spectrophotometer (Jenway, model 6505, UK). The existence of biomolecules in the shell of synthesized AgNPs was investigated using FT-IR (BRUKER, model TENSOR 27, Germany) analysis. Biosynthesis of AgNPs was demonstrated by x-ray diffraction (X'Pert PRO, SciSpec Co. Thailand) analysis. The shape of the freeze dried AgNPs was analyzed by SEM (Hitachi model S-4160, Japan). The particle size distribution and zeta potential analysis of biosynthesized AgNPs were evaluated via dynamic light scattering (DLS) and zeta potential analysis using a Malvern Zetasizer Nano range instrument (Malvern Instruments Ltd., Malvern, UK).

Plant sample and preparation of extract

Olea europaea (variety Sevillana) leaves were collected from Agricultural Research Garden, Khorramabad, Iran. Before the extraction, the leaves were dried in shadow, milled, homogenized and kept at 4 °C until analysis. The same sample was used in the whole optimization study.

To prepare the extract, 10 g of air-dried and pulverized olive leaves were extracted by ultrasonic bath for 24 h with 50 mL distilled water and filtered using Whatman filter paper. Finally, the filtrate was centrifuged for 10 min at 4,000 rpm. The supernatant was used for the synthesis of AgNPs.

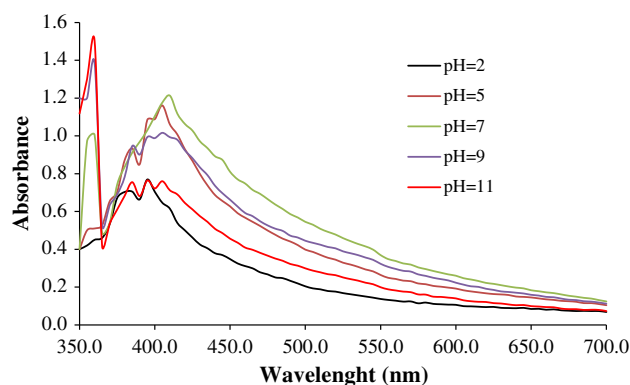


Fig. 1 Effect of extract pH on AgNPs synthesis

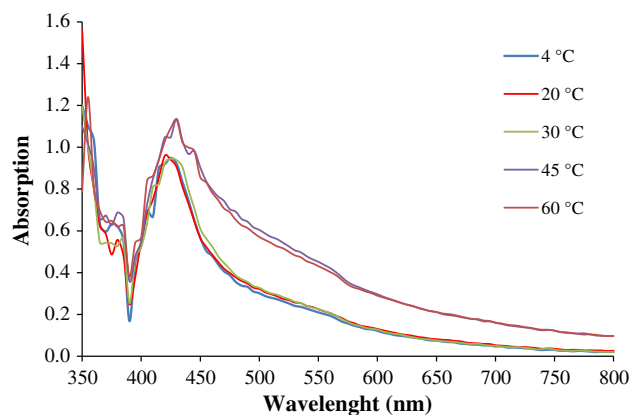


Fig. 2 Effect of temperature on AgNPs synthesis

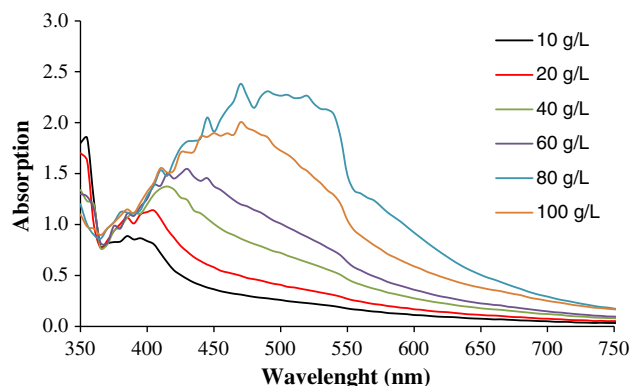


Fig. 3 Effect of extract concentration on AgNPs synthesis

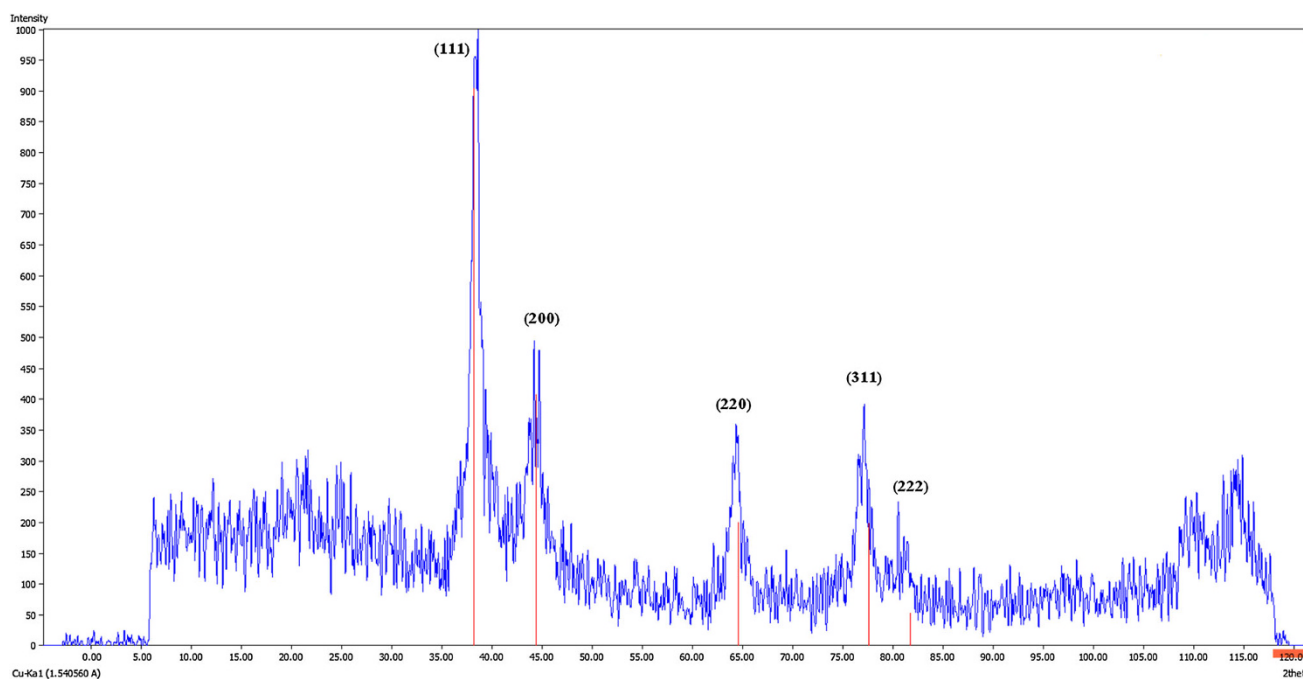


Fig. 4 XRD pattern of biosynthesized AgNPs (blue) and reference XRD pattern of cubic silver (red)

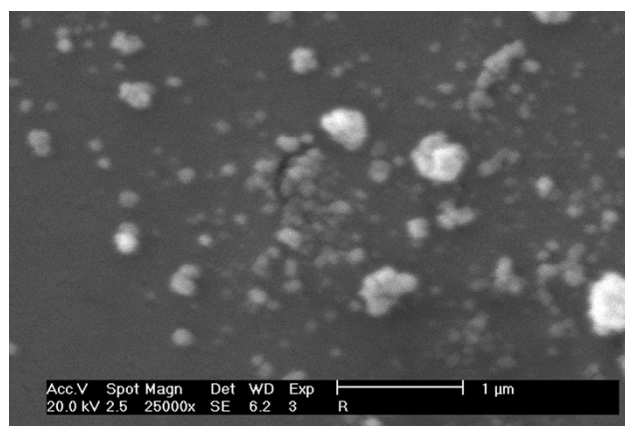


Fig. 5 SEM image of biosynthesized AgNPs

Biosynthesis of silver nanoparticles

40 mL (20 % w/v) of olive leaves aqueous extract, 10 mL of ammonia solution (1 M), and 10 mL of silver nitrate solution (10 mM) were mixed. The solution pH adjusts to the desired value using sodium hydroxide or phosphoric acid solution and then diluted until 100 mL with distilled water. The mixture was stirred for 4 h at 45 °C.

Isolation of AgNPs for characterization

After centrifuging of AgNPs solution for 10 min at 10,000 rpm, the AgNPs were sedimented at the bottom of the conical tube. The supernatant phase was removed and

AgNPs were washed with 10 mL water for three times. After washing, the residue was transferred to freeze dryer. Finally, the obtained powder was subjected to XRD, FT-IR, and SEM analysis.

In vitro cytotoxicity of synthesized AgNPs, and extract containing AgNPs

Cell viability was calculated according to the method developed by Denizot and Lang [24] using the reduction of 2-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan. MCF-7 and normal cells (human blood mononuclear cells) were treated with different concentration levels of biosynthesized AgNPs (dispersed in water) and dispersed AgNPs in olive leaves extract. The treated cells were incubated for 24 h at 37 °C for cell viability analysis. Finally, the treated cells were subjected to MTT assay. MTT stock concentration (5 mg/mL) was prepared in PBS, and 100 μL of this solution was added to each AgNPs-treated wells and incubated for 4 h. Then, 100 μL of dimethyl sulphoxide (DMSO) was added to each well, and the absorbance values were determined by spectrophotometry at 490 nm (Eliza MAT 2000, DRG Instruments, GmbH). Results were expressed as cell viability.

Data analysis

All measurements were performed in triplicate. The One-way analysis of variance (ANOVA) was done for

expressing experimental significance of results. In cell viability test, the p values <0.05 were considered as significant.

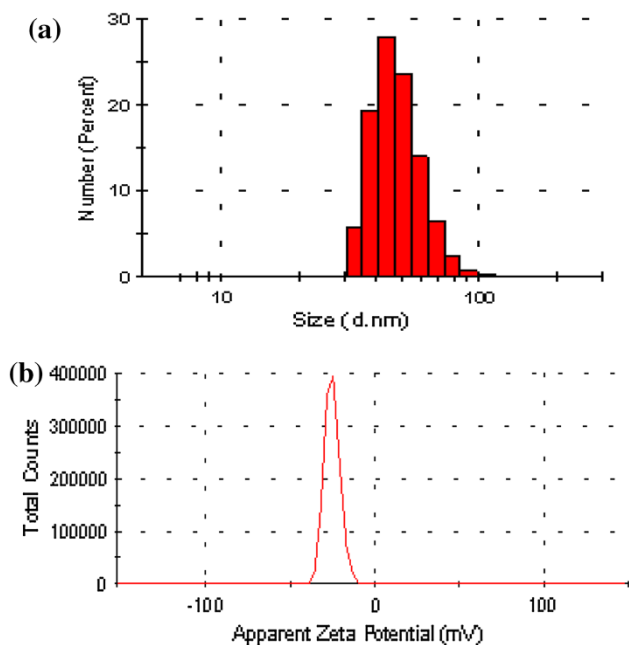


Fig. 6 DLS analysis (a) and zeta potential (b) measurement of biosynthesized AgNPs

Results and discussion

The main parameters affecting the formation of nanoparticles, including pH of extract, temperature of synthesis process and extract concentration were investigated and optimized.

Characterization of biosynthesized AgNPs

In biosynthesis of AgNPs using plant extracts, various constituents may contribute in the reduction process of silver ions. Therefore, changing the chemical state (e.g., ionization) of these constituents can be affected on performance and rate of reduction process. For this reason, the effect of extract pH on the synthesis of AgNPs in the range of 2–11 was investigated using UV–Visible spectrophotometer. The results (Fig. 1) show that the rate of AgNPs synthesis increases with increasing pH up to pH = 7 and then decreases. Therefore, pH = 7 was selected as optimum pH in further experiments.

Synthesis of silver nanoparticles was performed at different temperatures in the range of 4–60 °C. The results in Fig. 2 show that the efficiency of silver nanoparticles synthesis was constant at 45 and 60 °C. Therefore, 45 °C was selected as optimum temperature.

To complete the reduction of silver ions to silver nanoparticles, different concentrations of extract were mixed with a constant volume of silver nitrate (1 mM). The

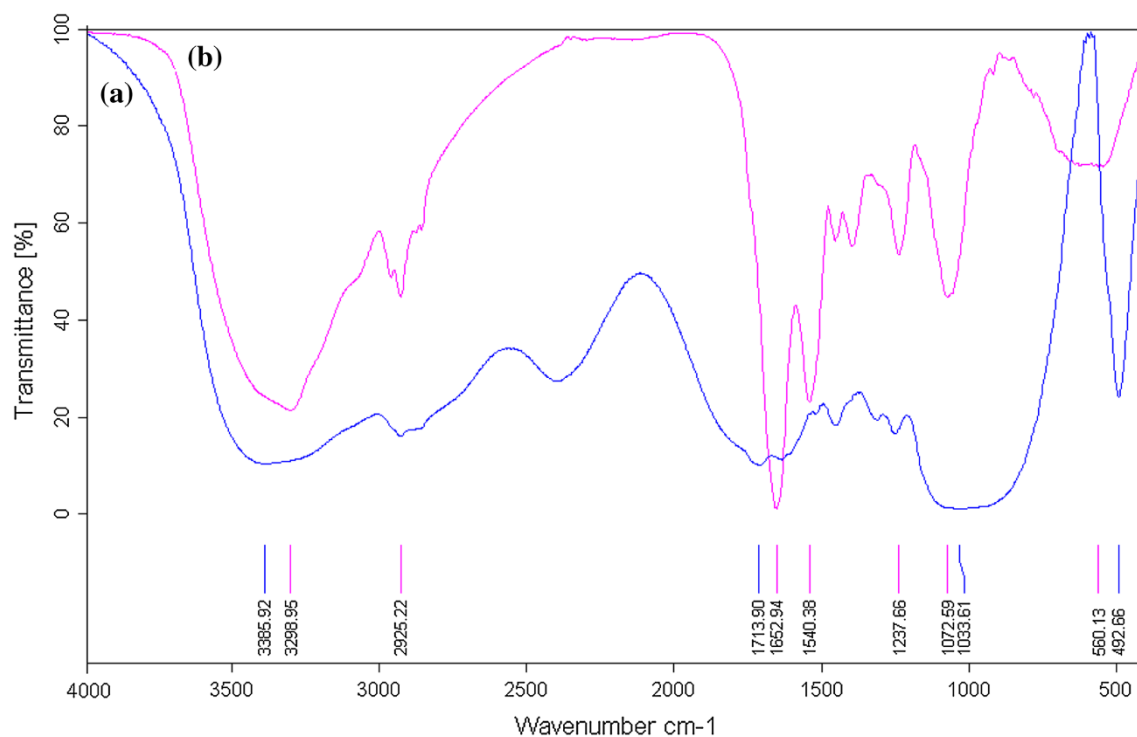


Fig. 7 FT-IR spectra of olive leaves extract (a) and biosynthesized AgNPs (b)



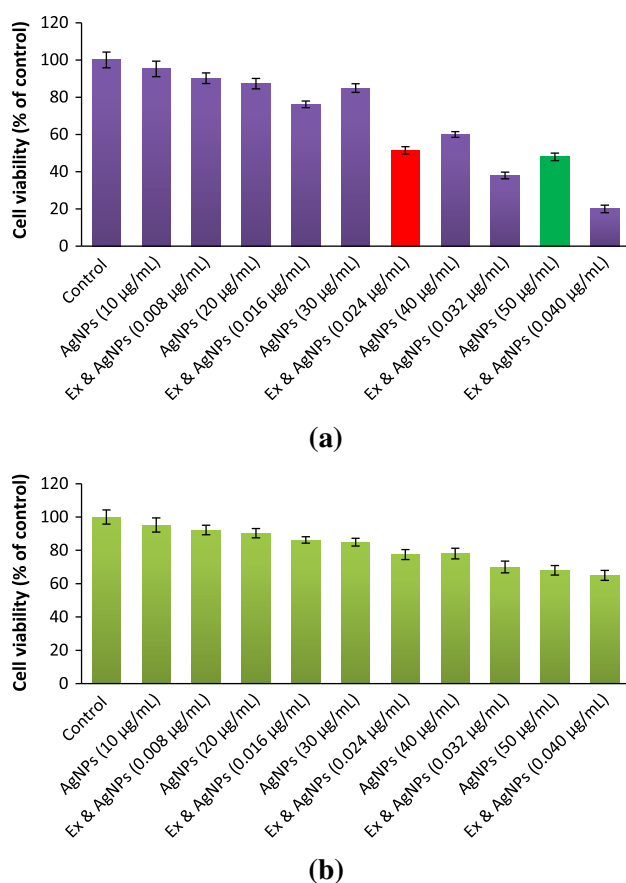


Fig. 8 MTT assay results. **a** Cytotoxic effects of dispersed AgNPs in olive leaves extract and AgNPs on cancer cell line (MCF-7) and **b** Cytotoxic effects of dispersed AgNPs in olive leaves extract and AgNPs on normal cell line (human blood mononuclear cells). Data are calculated as mean \pm RSD (relative standard deviation) of three experiments. AgNPs dispersed AgNPs in purified water, Ex and AgNPs dispersed AgNPs in the olive leaves extract. Percentage of cytotoxicity is expressed relative to untreated controls (*significant $p < 0.05$)

results in Fig. 3 show that increasing the concentration of extract led to synthesize more AgNPs until 8 % w/v and then decreases.

The XRD patterns of synthesized AgNPs are shown in Fig. 4. In comparison with cubic Ag reference pattern, the spectra data reveal the five diffraction lines (111), (200), (222), (311), and (222) which are compatible with the standard pattern.

The SEM image shows that the synthesized AgNPs are in spherical structures (Fig. 5). The results of DLS analysis showed the average size of nanoparticles is 90 nm (Fig. 6a). Zeta potential is an important parameter for understanding the state of the nanoparticle surface and predicting the long-term stability of the dispersion. According to previously reports, nanoparticles with zeta potential values greater than +25 mV or less than -25 mV typically have high degrees of stability.

Dispersions with a low zeta potential value will eventually aggregate due to inter-particle attractions. In the other hand, the zeta potential of nanoparticles strongly depends on pH and electrolyte concentration of the dispersion [14]. The zeta potential value of dispersed synthesized AgNPs in deionized water in the absence of any electrolyte was -25.3 mV (Fig. 6b).

Figure 7 shows the FT-IR spectra of dried olive leaves aqueous extract and synthesized AgNPs. Presence of similar peaks with small shift in both spectra reveals that the synthesized AgNPs are containing natural compounds from extract. Stability of AgNPs can be attributed to existence of these compounds in coating of nanoparticles.

In vitro cytotoxicity of biosynthesized AgNPs and extract containing AgNPs

In previous reports [14, 15], the biosynthesized AgNPs were isolated from the extract and their anticancer effects were examined. In this study, the anticancer activities of biosynthesized AgNPs were compared with extract containing AgNPs.

As observed from the Fig. 8a by increasing in concentration of AgNPs in olive leaves extract, cytotoxicity in MCF-7 cells was increased. The IC₅₀ value of dispersed AgNPs in extract was 0.024 µg/mL, whereas this value for isolated and dispersed AgNPs in purified water was 50 µg/mL. In agreement with previous reports [14, 15], AgNPs in extract and dispersed AgNPs in purified water have no significant cytotoxicity at their lower concentration on normal cell lines (Fig. 8b). The results show that the cytotoxic effects of biosynthesized AgNPs increased in the presence of extract on cancer cell line. This behavior can be attributed to the compounds in the extract that enhanced the activity of the AgNPs.

Conclusion

The results indicated anticancer activities of dispersed AgNPs in the olive leaves extract were higher than AgNPs on MCF-7 breast cancer cell line. Therefore, the olive leaves extract containing silver nanoparticles might be a potential alternative agent for human breast cancer therapy. The result of zeta potential shows that the biosynthesized AgNPs have a long-term stability.

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