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Green Synthesis of Silver Nanoparticles Using *Mentha aquatic* L Extract as the Reducing Agent

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Abstract

Background: Developing effective methods for the synthesis of bio-compatible and non-toxic nanoparticles is the main goal of nanotechnology. In the most chemical methods, a chemical reducing agent is used to reduce metal ions. But, in chemical methods, the stability of nanoparticles is controversial and synthesis in large sizes is much more difficult. Moreover, there is an increasing demand for the synthesis of nanoparticles using eco-friendly methods in order to avoid the harmful effects of chemical factors. Green synthesis is an alternative method for this purpose. The aim of this study was to assess green synthesis of silver nanoparticles using *Mentha aquatic L* extract as the reducing agent.

Methods: In this Study, *Mentha aquatic L* was used as a reducing agent for the synthesis of bio-compatible silver nanoparticles. Green synthesis of silver nanoparticles was performed in three phases: preparing *Mentha aquatic L* extract, preparing silver nitrate solution, and adding the extract to the silver nitrate solution. Morphology of the sample and particle size distribution were analyzed by scanning electron microscopy (SEM) and dynamic light scattering (DLS), respectively. Light absorption of nanoparticles was analyzed by Ultraviolet - visible (UV-Vis) absorption spectroscopy.

Results: Analysis of UV-Vis absorption at 430 nm indicated the surface plasmon resonance (SPR) property of the particles. Considering the peak of dynamic light scattering (DLS) plot, particle size distribution of silver nanoparticles was below 100 nm. According to the results of scanning electron microscopy (SEM), nanoparticles with mean particle size of 30 nm and spherical shape were the most frequent particles. The Energy-dispersive X-ray spectroscopy (EDX) confirmed the presence of silver.

Conclusion: Based on the results, proper synthesis of nanoparticles with uniform distribution was confirmed. Therefore, *Mentha aquatic L* extract is an effective reducing agent that can be used as an alternative for chemical products in the synthesis of nanoparticles.

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Introduction

Silver is one of the most widely used metals in nanotechnology and it has many applications in medicine, electronics, optics, and space industries (1, 2). Antibacterial, antifungal, antiseptic, antiviral, and anti-angiogenic properties of this metal have made it as a useful agent in medicine (3, 4). Today, special interest has been directed to the green synthesis of nanoparticles and a variety of biologic systems has been used for this purpose (5, 6). For example, using plants in the synthesis of nanoparticles, due to avoiding toxic and pollutant substances, is an eco-friendly method (7). Reduction of metal ions in solution and actually their neutralization is the base of nanoparticles synthesis (8). Plants are the main economic and effective sources for the synthesis of nanoparticles (8). Studies show that some medicinal herbs and natural products, generally used by people, have biological and pharmaceutical effects (9). Since medicinal herbs do not have chemical harmful effects and have beneficial effects against some pathogens and bacteria, they can be used as antiseptic, antiviruse, antioxidant, and antimicrobial agents (10).

Many studies have been performed on synthesis of gold and silver nanoparticles using plants extracts (11). According to the studies on green synthesis using bio-compatible agents, the main advantage of using plants for nanoparticles synthesis is that this method is bio-secure, easy to use, and includes a wide variety of metabolites that are involved in ion reduction process (12). Green synthesis of nanoparticles using plants such as Hindi grape (13), Cinnamon (14), Goosefoot (15), and lemon extract (16) has been reported so far.

Since *Mentha aquatica* L is easily available and its extract has a good potential to reduce ions, it was used in this study. *Mentha aquatica L* is widely used in cooking, cosmetics, and medicine. *Mentha aquatica* L (from the family Lamiaceae or Labiatae) originally grows in Europe but it can grow in most climate zones. *Mentha aquatica* L, as its name implies, grows in water and especially at the edge of rivulet, rivers, water channels, and in humid regions.

Antibacterial effects of *Mentha aquatica* L extract on a variety of bacteria have been evaluated and verified. Previous studies show that this plant, with its medicinal and biological effects, has been used as an antioxidant in traditional medicine (17). High levels of phenol and flavonoid in this plant have also been reported (18). Since, nanotechnology is very expensive due to the high costs of synthesis and identification of the materials structure, through using herbal agents in the synthesis of nanoparticles, the costs can be greatly reduced.

Synthesis of nanoparticles requires three materials including metal ions, reducing agents, and protective agents that all of them are chemicals. On the other hand, given the importance of medicinal herbs used in the traditional and modern medicine and some advantages of *Mentha aquatica* L (20) including having minimum side effects, availability due to wide production of this plant in some areas of Iran, especially in North of Iran and being cost-effective and bio-compatible, a special turning towards using *Mentha aquatica* L for the synthesis of silver nanoparticles has been reported. The main aim of this study was to synthesize silver nanoparticles using *Mentha aquatica* L extract as the reducing agent of Silver Nitrate (AgNO3).

Materials and Methods

Production, Drying, and Extraction: In this study, *Mentha aquatica* L grown in the North of Iran (natural habitat of Mazandaran, Sari, central district) was used after being identified and approved by an expert in plant systematics (21). The leaves and aerial parts of the plant were exposed to air in the shade and away from direct sunlight to be dried. Then, they were powdered by electric mill powder. Afterwards, a certain amount of the powder was poured in a flask and twice-distilled water was added to the flask. Then, the mixture was boiled. Finally, using a filter paper (pore size of 25 microns), the extract was filtered and poured in the Falcon tube and kept in the refrigerator for later use (22, 23).

Silver Nitrate Solution (AgNO3): In this study, Silver Nitrate (AgNO3) (purchased from Merck Co., Germany) was used (24, 25). Considering the stoichiometry calculations and molar mass of the solution, Silver Nitrate solution 3mM was prepared in order to synthesize silver nanoparticles and in order to reduce the silver ions, it was placed at room temperature and in dark condition while it was centrifuging at 500 rpm (24, 25).

The Synthesis of Silver Nanoparticles: In order to prepare a medium with pH 11, NaOH 1mM was added to silver nitrate solution drop by drop. Then, the extract was added to the solution of silver nitrate while it was being mixed. Any change in the color of the solution indicates the reaction and synthesis of silver nanoparticles. When nanoparticles were synthesized, they were centrifuged at 20000 rpm and isolated from the aqueous phase and rinsed again. Nanoparticles were centrifuged and rinsed until reaching pH 7. Finally, the synthesized silver nanoparticles were placed in the vacuum oven at 80˚C to be dried (24 and 26).

Results

According to theUV-Vis spectrum that compares the absorption of the two media including the extract (A) as the control sample and silver nanoparticles (B), medium containing silver nanoparticles had the maximum absorption at 430 nm due to the interaction of light with the surface of silver nanoparticles. This interaction indicates the existence of surface plasmon resonance (SPR) (Figure 1).

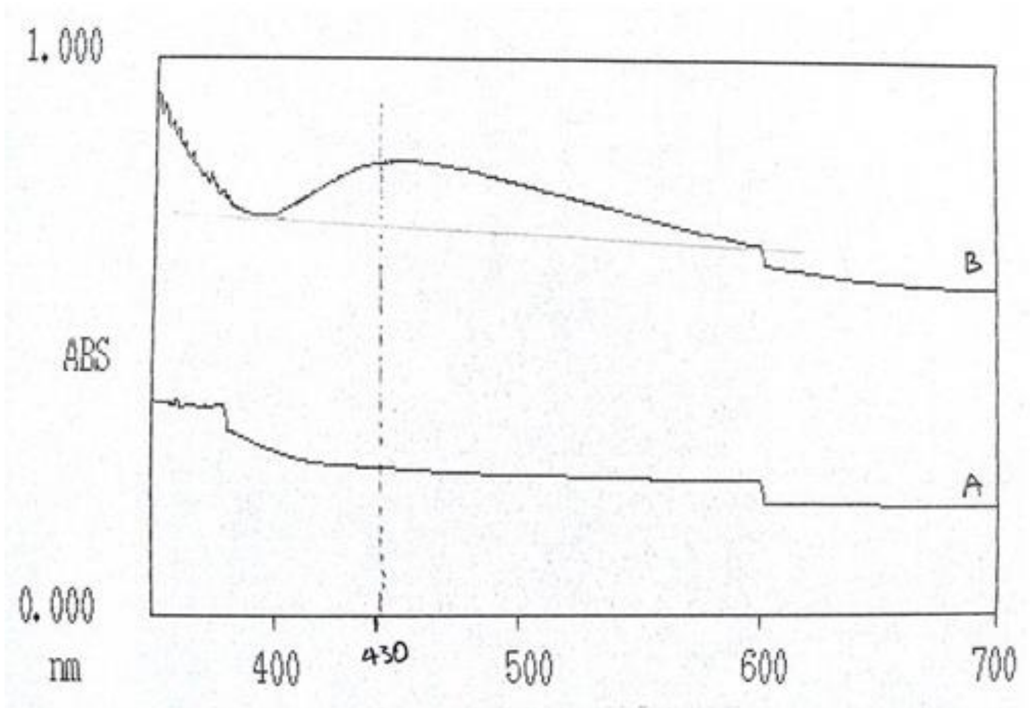

Figure 1. *Absorption of UV-Vis by the extract (A) as the control sample and by silver nanoparticles (B).*

Analysis of dynamic light scattering (DLS) plot of silver nanoparticles showed particle size distribution of below 100 nm (Figure 1). Mean particle size was 38.42 nm and the PDI was 0.2 (Figure 2).

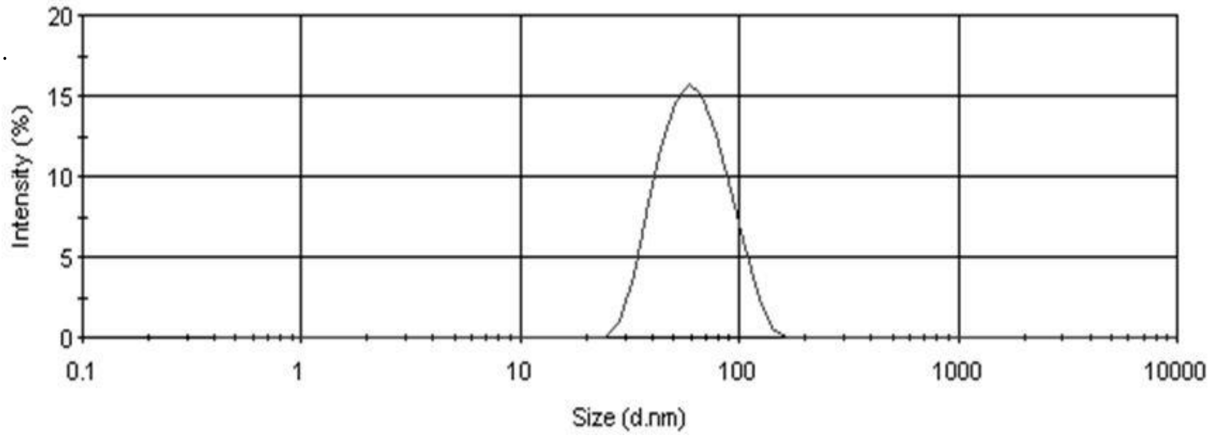

Figure 2. *Dynamic light scattering (DLS) of silver nanoparticles*

According to the scanning electron microscopy (SEM) silver nanoparticles with mean particle size of 30 nm and spherical shape were the most frequent particles (Figure 3).

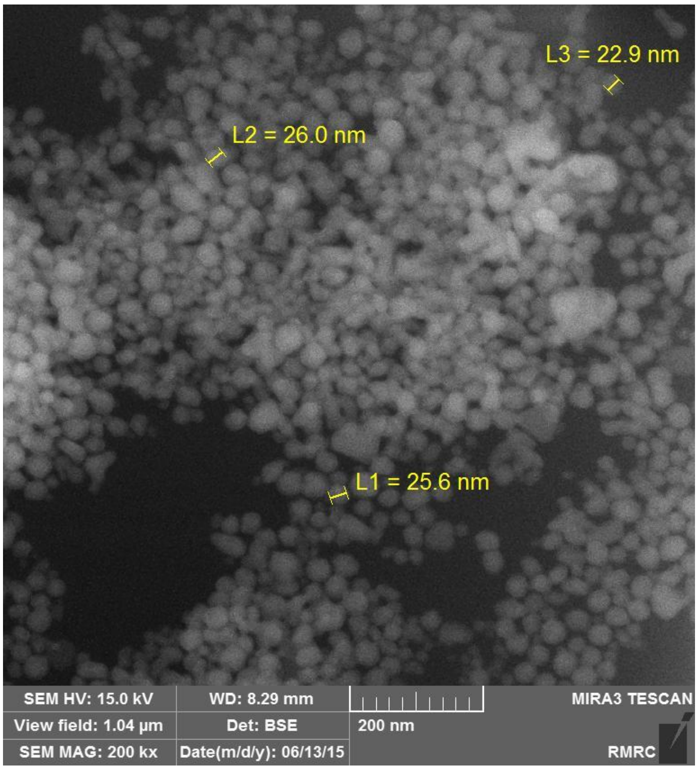

Figure 3. *Scanning electron microscopy (SEM) of silver nanoparticles*

The two sharp peaks (AgLα and AgLβ) in Energy-dispersive X-ray (EDX) also confirmed the presence of silver in the synthesized samples (Figure 4).

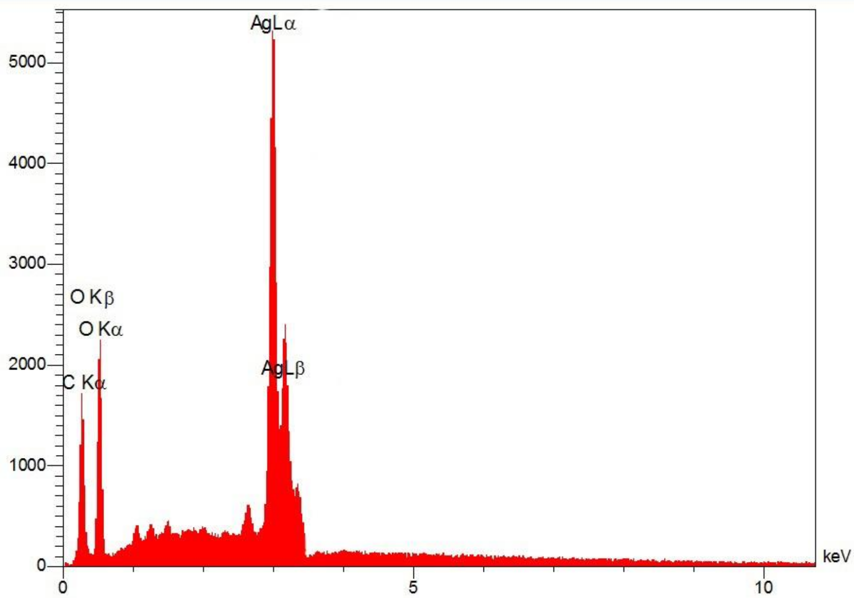

Figure 4. *Analysis of silver nanoparticles EDX with its two sharp peaks (AgLα and AgLβ)*

Discussion

Due to the special properties and applications of nanoparticles in biological fields and pharmaceutical industries, synthesis of nanoparticles (10-100 nm) has attracted the attention of many researchers (27,28). Nanotechnology has great potential to be developed, but it also concerns researchers over the probable risks to human health and environment. Therefore, in this study, by employing green chemistry technology for the synthesis of nanoparticles, Mentha aquatic L was used as a reducing agent. Green synthesis, through using natural reducing agents of Mentha aquatic L extract, has significantly lower toxicity. Furthermore, several studies performed on medicinal herbs and natural products, have confirmed that plants, due to anti-fungal, anti-septic, anti-virus, antioxidant, and antimicrobial properties, can be used in medicine production (29).

The main aspect of nanotechnology is developing bio-compatible and non-toxic methods for the synthesis of nanoparticles. Most chemical methods use a chemical reducing agent (e. g, Sodium Borohydride) to reduce metal ions and a stabilizer (e. g, Polyvinylpyrrolidone) to control the growth of particles and to prevent aggregation. For these reasons, there is an increasing demand for the synthesis of nanoparticles using bio-compatible methods. Synthesis of nanoparticles using green synthesis is an alternative method (30). Metal nanoparticles with their special use in various fields such as catalyzers, optoelectronic, biologic indexers, and medicine have attracted the attention of many researchers (31). A great number of these applications are related to Silver nanoparticles that have been produced in this study through a biosynthesis method (32). In a study performed by Nikbakht et al (2015), the biosynthesis of silver nanoparticles using aqueous and methanol extracts of jujube, and its antibacterial effect were investigated. The results showed that biosynthesis of nanoparticles using plants extracts can enhance their medicinal effects and it was also revealed significant increase of the antibacterial effect of the extract consisting of nanoparticles (24). In another study performed by Chahar dooli et al (2014), the synthesis of silver nanoparticles using biologic methods and Oak extract, and its antibacterial effect on hospital infection pathogens were investigated. Silver nanoparticles were synthesized in a short-time phase and their antimicrobial effects were also confirmed (26).

In the present study, scanning electron microscopy (SEM) showed uniform size of the synthesized nanoparticles (20-30 nm). The results of the present study are consistent with the results of Shankar et al (2004) study on the synthesis of silver nanoparticles using Azadirachtaindica. In the mentioned study, the size of nanoparticles was 5-35 nm and they were spherical (32). Ahmad et al (2010) (34) and Chandaran (2006) (35) have respectively reported 10 and 15 nm for the size of Silver nanoparticles.

In another study performed by Shams et al (2015), silver nanoparticles were synthesized using bitter olive. The study was resulted in the synthesis of hexagonal nanoparticles with mean size of 20 nm (36). In Mohaseli et al (2015) study, analysis of UV-Vis spectrum showed a peak at 420 nm that indicated nanoparticles biosynthesis in the extract, and analysis of TEM showed spherical shape and mean size of about 14 nm for nanoparticles (37). In the other study performed by de Sousa Barros et al (2015), chemical compositions and properties of the extract of various species of Mentha were evaluated and anti-fungal and antioxidant properties were reported for the studied species (38).

Conclusion

According to the results of the present study and since Mentha aquatic L is a cost-effective, available and useful plant in medicine, it can be used as the best choice for the biosynthesis of nanoparticles. Among methods of nanoparticles synthesis, biosynthesis is considered to be the cost-effective, safe, and bio-compatible one.

Since in this method, Silver nanoparticles are synthesized without using hazardous chemicals, they can be used in the health-related industries such as healthcare and the environment. This study showed successful synthesis of silver nanoparticles using the extract of Mentha aquatic L. This method is a cost-effective and rapid method that can be applied at room temperature.

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