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An investigation of the optimal conditions for the green synthesis of silver nanoparticles using an aqueous extract from the plant *Agrimonia eupatoria L.* †

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Abstract:

In this research, –silver nitrate and an aqueous extract of the plant *Agrimonia eupatoria L.* were used for the synthesis of silver nanoparticles (AgNPs). The optimal conditions for this green synthesis were examined: the concentration of starting substances, pH value, and temperature. The best conditions for the most increased AgNPs yield production were a 5 mM concentration of $AgNO_3$, 1% concentration of extract, a reaction temperature of 25 °C, pH=6, and a reaction time of 3 h for synthesis.

Keywords: Silver Nanoparticles, Green Synthesis, *Agrimonia eupatoria L.*

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1. Introduction

Physical, chemical, and biological methods were used for the synthesis of metal nanoparticles (MtNP). Physical and chemical methods for MtNPs synthesis have many drawbacks including the use of expensive equipment, high energy consumption, and the use of toxic chemicals, which pose an environmental problem [1-3]. There has been a need for an environmentally friendly alternative to synthesizing MtNPs, the focus of which is the green synthesis of MtNPs using plants, microorganisms, enzymes, polysaccharides, and biodegradable polymers [3-4]. Organism extracts can be used both as reducing agents and as stabilizers in the synthesis of nanoparticles. Innovative and diverse applications of MtNP in different fields of medical science, environmental science, and agriculture, led to the accelerated development of different ways of synthesizing these compounds during the last [1, 5].

A silver nanoparticle (Ag NP), as stable, colloidal dispersion in water or organic solvents, is most commonly prepared by chemical reduction in organic solvents or water. A plant extract can be used as a reducing agent and as a stabilizer (to prevent unwanted agglomeration of colloids) during nanoparticle synthesis [6]. Nanoparticles of silver have unique physical, chemical, and biological properties. There is a significant catalytic and antibacterial activity in these nanoparticles, as well as good potential for nanobiotechnological applications [7].

Agrimonia eupatoria L. (common name: *agrimony*) belongs to the family *Rosaceae* (Tribe: *Sanguisorbeae*). The plant is known for being used as a raw material for the extraction of medicinal ingredients or the production of medicines in the pharmaceutical industry. The plant has antioxidant and antibacterial properties, but also anti-inflammatory, neuroprotective, antidiabetic, hepatoprotective, and anticancer properties [8]. As part of our earlier research, silver nitrate and acetone extract of *A. eupatoria L.* were used for the synthesis of silver nanoparticles [9]. Our study examined the best conditions for the synthesis of silver nanoparticles from *A. eupatoria L.* aqueous extracts.

2. Materials and Methods

Silver nitrate, sodium hydroxide and nitric acid used were from Sigma Aldrich, USA. All solutions were prepared in distilled water. The aqueous extract of the plant was prepared according to a previously published procedure [8]. Dried, crushed plant material was extracted in distilled water by maceration. In brief, 60 g of the plant material was soaked in 800 mL of the solvent. The plant material was macerated three times at room temperature using a fresh solvent every 24 hours. After every 24 hours, the samples were filtered through a filter paper and the filtrates were collected and evaporated to dryness using a rotary evaporator (DLAB, RE 100 S) at 40 ^{0}C. All nanoparticle synthesis reactions were performed on a magnetic stirrer (MAGE 12/17) under controlled conditions. Perkin Elmer Lambda365 spectrophotometer was used to monitor the synthesis of AgNPs in the wavelength range 200–800 nm. DM0412 MicroCentrifuge from Scilogex | Lab. Equipment is used for centrifugation of suspensions at 4 500 rpm for 20 min. The data were analyzed using OriginPro 2019b -64bit software.

The optimal conditions for this green synthesis were examined: the concentration of starting substances, pH value, and temperature [10]. Silver nitrate was dissolved in concentrations of 5 mM, 10 mM, and 20 mM. The pH of the reaction mixtures was adjusted to 4, 6, and 8 using solutions of 0.1 M NaOH and 0.1 M HNO_3. The reaction mixture was heated to 25°C and 50°C on a magnetic stirrer under controlled conditions. Visual color change (from light yellow to dark brown) and UV-Vis s spectrophotometry were used to monitor the process of AgNPs formation. After the synthesis of AgNPs, the suspensions were centrifuged at 4 500 rpm for 20 min. Obtained residue after centrifugation resuspended in demineralized water, centrifugated again, and the precipitated nanoparticles were then dried in a hot air oven (40 °C) and stored at 4 °C.

3. Results

In this research, we used silver nitrate and an aqueous extract *A. eupatoria L*. for the synthesis of silver nanoparticles (AgNPs).

3.1. UV-Vis spectral analysis

The generation of AgNPs in solution during their synthesis using extracts was monitored spectrophotometrically. The color change of solutions from light yellow to dark brown is a characteristic indicator r of the synthesis AgNPs. The color change is a result of the surface plasmon resonance (SPR) effect. The UV-Vis absorption spectra of formed nanoparticles were recorded (200–800 nm) and the highest peaks were positioned within 425–475 nm (characteristic peak for AgNPs), suggesting the formation of AgNPs (Figure 1). The maximum absorption values during biosynthesis were obtained at 3 h, and thereafter the absorption peaks did not increase, thus indicating the end of the synthesis process. Then, the influence of the concentration of AgNO3, temperature, and pH on the biosynthesis of nanoparticles using A. *eupatoria* aqueous extracts was evaluated. The initial conditions for the synthesis of both types of nanoparticles were 5 mM $AgNO_3$, 25 ^{0}C, and 1% extract concentration without adjustment of pH values (pH ≈6).

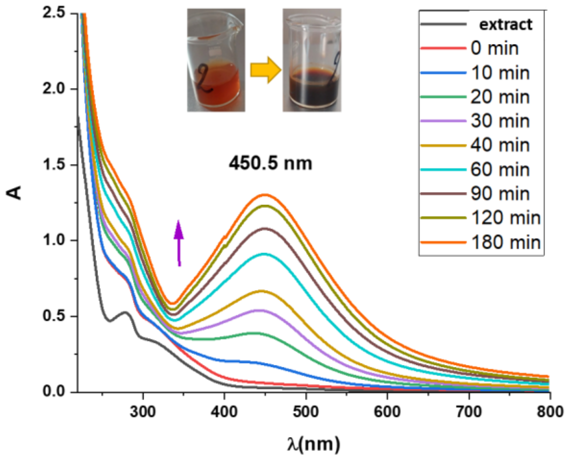

Figure 1. Time-dependent UV-Vis absorption spectra and color change of biosynthesized AgNPs using aqueous extract *A. eupatoria*

3.2. Temperature dependence

The conditions for testing the temperature sensitivity were 5 mM $AgNO_3$, 50 ^{0}C, and 1% extract concentration without adjusting the pH value. The reaction mixture was heated to 25°C and 50°C on a magnetic stirrer under controlled conditions. When the temperature was increased to 50 ^{0}C, the reaction accelerated significantly.

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3.3. pH dependence

The conditions for testing the pH sensitivity were 5 mM $AgNO_3$, 25 ^{0}C, 1% extract concentration. To adjust the pH= 4, a few drops of 0.1M HNO_3 solution were added to the mixture of extract solution and $AgNO_3$. The value of pH=6 - has a solution obtained by mixing a solution of extract and silver nitrate. To adjust pH=8, a few drops of 0.1 MNaOH solution were added. Figure 2 shows the UV-Vis absorption spectra of AgNPs as a function of the npH value. According to the results, it can be concluded that pH =6 is the most optimal value for the synthesis of AgNPs with the help of this plant.

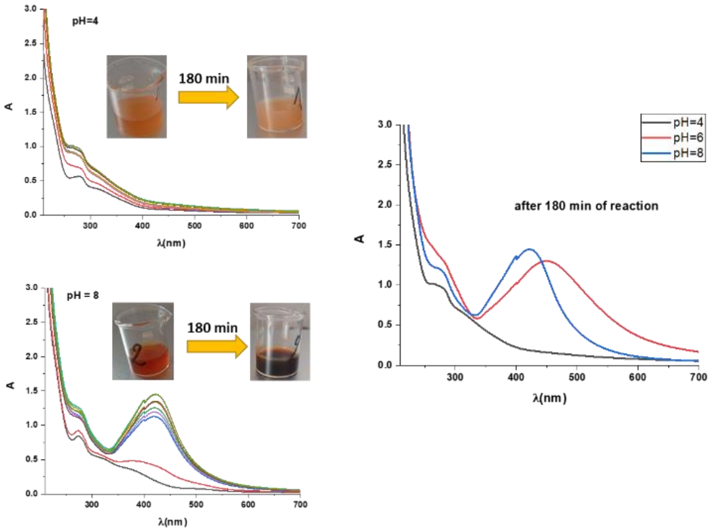

Figure 2. UV-Vis absorption spectra of AgNPs as a function of npH value

3.4. Concentration dependent

Initially, 25°C, 1% extract concentration, and pH=6 conditions were used to examine the dependence on $AgNO_3$ concentration. Three extract solutions were prepared to which $AgNO_3$ was added in concentrations of 5 mm, 10 mm, and 20 mm. As seen in Figure 3, 5 mm $AgNO_3$ is the optimal concentration for AgNPs synthesis.

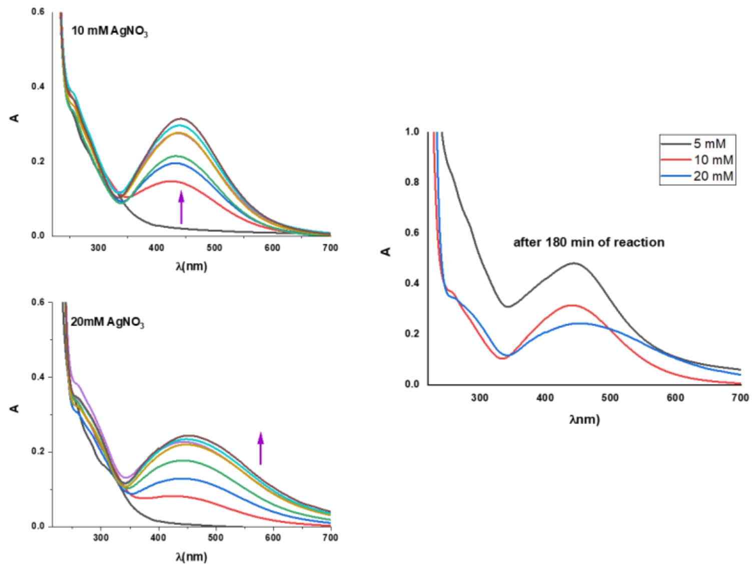

Figure 3. UV-Vis absorption spectra of biosynthesis AgNPs as a function of concentration $AgNO_3$

4. Conclusions

According to the results of this research paper, *A. eupatoria* is good choice for the green synthesis of silver nanoparticles. Plant's aqueous extract is an effective reducer and stabilizer of nanoparticles. The time-dependent UV-Vis absorption spectra and the color change of the reaction solution are indications of the gradual synthesis of silver nanoparticles. By increasing the temperature, the rate of biosynthesis of silver nanoparticles increases obviously increases. By examining the optimal pH values for this biosynthesis, it was concluded that acidic environments are suitable, and the most optimal pH value is 6, which is achieved by simply mixing starting substances. By examining this reaction at different concentrations of $AgNO_3$, it could be concluded that this synthesis is best with a concentration of $AgNO_3$ of 5 mM. And finally, it could be concluded that the best conditions for obtaining the highest yield of AgNPs are $AgNO_3$ concentration of 5 mM, a reaction temperature of 25 ^{0}C, pH 6, and a reaction time of 3 h for synthesis.

Supplementary Materials:

Author Contributions: Conceptualization, Z.M. and A.K.; methodology, K.M.; software, M.G.; validation, Z.M., A.K. and K.M.; formal analysis, M.G.; investigation, A.K.; resources, K.M.; data curation, A.K.; writing—original draft preparation, A.K.; writing—review and editing, M.G.; visualization, A.K.; supervision, Z.M.; project administration, K.M.; funding acquisition, Z.M. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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