

Original Article: Green, Rapid and Facile Synthesis of Silver Nanoparticles Using Extract of *Stachys Lavandulifolia* Vahl and Study of the Effect of Temperature, Time, Concentration, and pH Parameters



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ABSTRACT

Today, nanotechnology is widely used in science and technology is growing rapidly. Nanoparticles synthesized with the help of plant extracts, due to the lack of use of external chemical agents, can have fewer side effects in medical applications. In this article, we synthesize silver nanoparticles (AgNPs) using the aqueous extract of *Stachys lavandulifolia* vahl. Plant extract in the synthesis of nanoparticles acts as a reducing and stabilizing agent and can also have fewer side effects in medical use and is environmentally friendly. The average size of nanosilver synthesized by this method is 68-69 nm and factors such as temperature, concentration, pH, and time were investigated to optimize the reaction. The formation of silver nanoparticles was confirmed by UV-Vis spectroscopy, Energy Dispersive Spectroscopy (EDS) analysis, X-ray diffraction (XRD), and Field Emission Scanning Electron Microscopy (FESEM).

Introduction

Nanotechnology is expected to underpin many of the major research and development innovations of the 21st

century. Plant extracts can act as a reducing and stabilizing agent in nanoparticle synthesis.[1]. The ability of plant extracts to reduce metal ions has been known since 1900. Although the nature of the reducing agents involved in these

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reactions is not well understood, the simple use of plants (tissues and plant extracts) to reduce metal salts to nanoparticles has attracted the attention of researchers. Compared to the use of chemical methods, plant extracts have received more attention and focus. In recent years, many biological processes have been used to synthesize silver nanoparticles. The existence of different compounds such as proteins, polysaccharides, polyphenols, terpenoids, and organic acids such as citrates, etc. with different concentrations cause the use of plant extracts as organic reducing agents [2].

Hence, nano-silver can be used to remove harmful impurities from an aqueous solution. Also, silver nanoparticles have advantages such as ease of use, being environmentally friendly, Non-toxicity, cheapness, and having a wide variety of metabolites that are involved in ion reduction. Nanoparticle biosynthesis as one of the most prominent intersections of nanotechnology due to the growing need for the development of environmentally safe technologies in the synthesis of materials, has attracted increasing attention [3]. Silver nanoparticles have been able to have different applications in recent years due to their antibacterial activity. They are important antibacterial agents against a wide range of antibiotic-resistant bacteria. According to previous studies, it has been reported that silver nanoparticles have antifungal activity [5], anti-inflammatory effects [6] anti-viral activity [7] anti-angiogenesis activity [8], and anti-platelet activity [9]. Today, a variety of plant extracts are used to synthesize silver, copper, and gold nanoparticles, such as broth extracts of neem [10], Aloe Vera [11], tamarind [12], Avena sativa [13], wheat [14], alfalfa [15], germanium [16], lemongrass [17] and tamarind [18]. Nanosilver particles were synthesized by cellulose along with glucose in alkali media [19]. Green synthesis of Cu/Cu₂O nanoparticles was carried out using an extract of the flowers of *Stachys lavandulifolia* for the first time by Mehrdad Khatami and his coworkers [20]. In another work, silver nanoparticles on *Stachys lavandulifolia* extract modified magnetic iron oxide nanoparticles as an antibacterial agent and their 4-nitrophenol catalytic reduction

activity were prepared [21]. In this study, we report the synthesis of silver nanoparticles by reducing the aqueous solution of silver nitrate through *Stachys lavandulifolia* vahl extract and determine the optimal conditions for the synthesis of this silver nanoparticle according to the parameters of pH, temperature, time, and concentration. Here we are going to introduce a green and efficient method in the biosynthesis of silver nanoparticles in the hope of the attention of biochemists and nanochemists.

Experimental

Preparation of extracts

Stachys lavandulifolia vahl was collected from Bojnourd city of North Khorasan province. To prepare the aqueous extract, the first 30 grams of the studied plant was washed and placed at room temperature to dry completely. This amount of plant was then poured into a 250 mL Erlenmeyer flask and 100 mL of distilled water was added. The mixture was boiled for 10 minutes. The aqueous extract was filtered using Whatman filter paper and the sample was centrifuged at 9000 rpm for 10 minutes to remove suspended particles from the extract. The extract was then poured into a falcon and kept at 4 °C for later use.

Synthesis of silver nanoparticles

For biosynthesis of silver nanoparticles, the first 5 mL of the prepared extract was slowly added to 0.001 M silver nitrate solution and sonicated for 15 minutes at 30 °C. The resulting colloidal solution was centrifuged at 9000 rpm for 20 minutes. The supernatant was then discarded and the centrifugation was repeated 3 times with the addition of deionized water to wash and disperse the precipitated nanoparticles. After each centrifugation, the supernatant was separated and deionized water was added to the precipitate. After centrifugation, the remaining suspension was placed on a petri dish to dry

Characterization of silver nanoparticle

Due to the reaction of silver nitrate with plant extract, the color of the solution changed to a

light-dark brown, which is a clear sign of the formation of silver nanoparticles [22]. This color occurs due to the stimulation of surface Plasmon vibrations in silver nanoparticles [23].

When the extract was mixed with an aqueous solution of silver nitrate, it began to change color from yellow to brown due to the reduction of silver ions, indicating the formation of silver nanoparticles (Figure 1).



Figure 1. Color changes of *Stachys lavandulifolia* vahl extract (a) before reaction and after reaction with silver nitrate (AgNO_3)

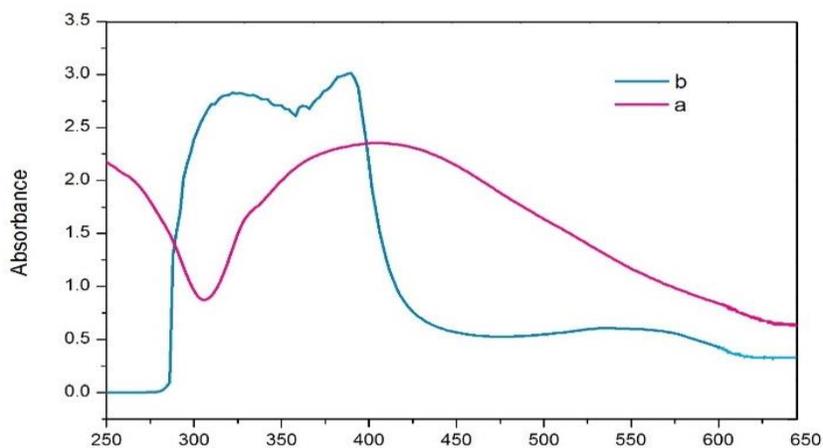


Figure 2. UV-Vis spectra of *Stachys lavandulifolia* extract (a) and Silver nanoparticles synthesized (b)

As we know, UV-Vis spectroscopy can be used to detect the shape and size of nanoparticles in aqueous suspensions [24]. The UV-Vis spectrum of reaction was shown in Figure 2. At about 450 nm, the maximum absorption is observed, which seems to be related to the synthesis of silver nanoparticles. The silver nanoparticles produced are between 68-69 nm.

EDS is used for the identification and characterization of the structure of the silver nanoparticles. EDS spectrum of the synthesized silver nanoparticles by *Stachys lavandulifolia* vahl extract is shown in Figure 3.

This result was confirmed by analysis of the XRD for the synthesized silver nanoparticles is shown in Figure 4.

The size and morphology of the synthesized silver nanoparticles from *Stachys lavandulifolia* vahl were determined by FESEM image and they are shown in Figure 5.

FTIR studies were carried out to identify the biomolecules responsible for the stabilization of the synthesized silver nanoparticles as well as the surface properties of the silver nanoparticles.

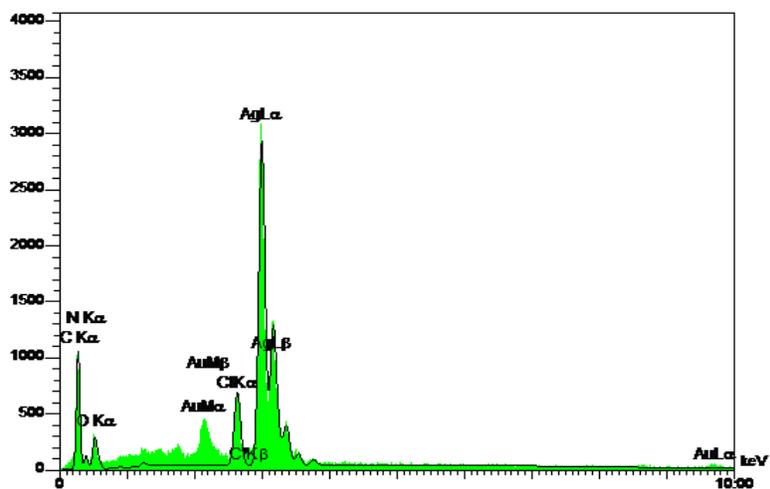


Figure 3. EDS spectrum of the synthesized silver nanoparticles

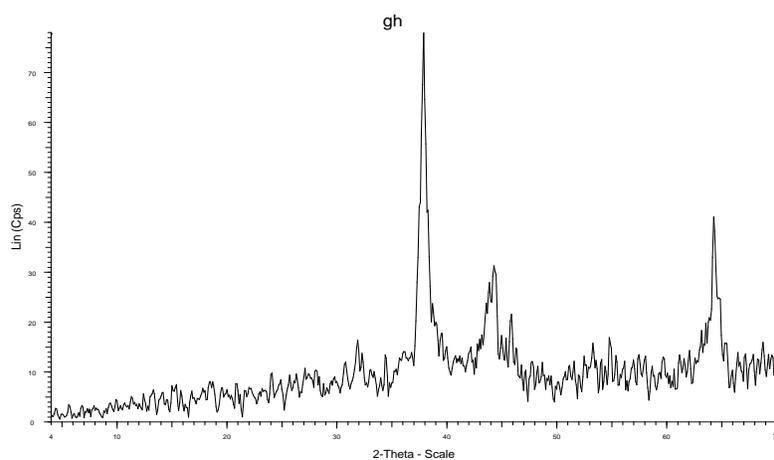


Figure 4. XRD spectrum of silver nanoparticles

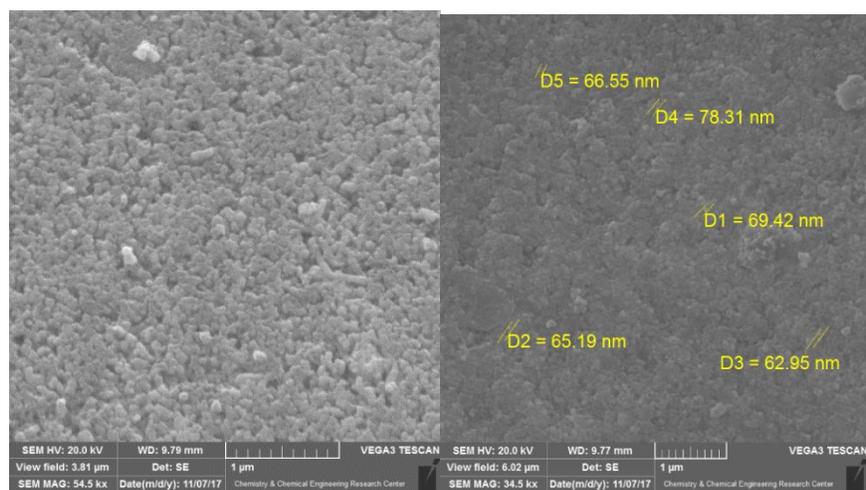


Figure 5. FESEM image of synthesized AgNPs

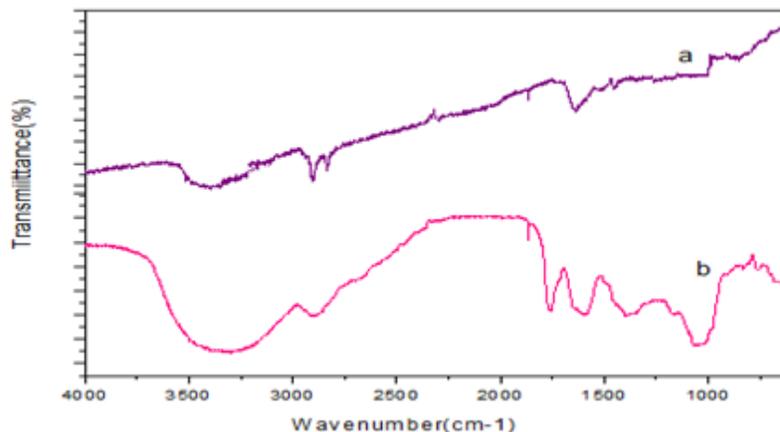


Figure 6. FTIR spectrum of (b) *Stachys lavandulifolia* vahl medicinal plant as raw and (a) after reduction of silver nanoparticles

Raw *Stachys lavandulifolia* vahl extract showed intense peaks at 3350, 2935, 1750, 1520 and 1156 cm^{-1} relative shift in position and intensity distribution were confirmed by FT-IR (Figure 6a and 6b). Figure 6a. shows the FTIR spectrum of *Stachys lavandulifolia* vahl as raw and after reduction of silver nanoparticles. The observed shifts, as well as intensity changes of bands in (Figure 6b) can be attributed to the binding of the C=O group with nanoparticles.

study of the effect of silver nitrate concentration on the synthesis of silver nanoparticles:

To investigate the effect of silver nitrate concentration, silver nanoparticles with different concentrations (0.012, 0.025, 0.037 and 0.052 M) was synthesized from silver nitrate solution. As can be seen in Figure 7, increasing the concentration of silver nitrate in λ_{max} has a negative effect. λ_{max} , the different graphs are about 435 and the same, on the other hand, λ_{max} is directly related to the size of nanoparticles. Therefore, increasing the nitrate concentration has little effect on the nanoparticle size.

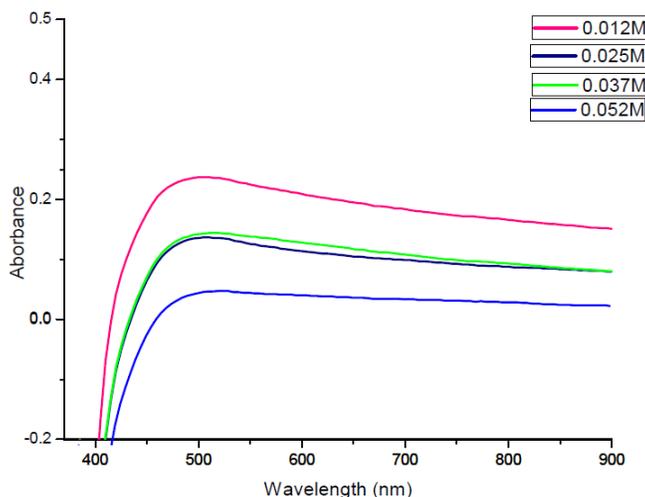


Figure 7. Effect of silver nitrate concentration on the synthesis of silver nanoparticles

Therefore, we can say that by increasing the concentration of silver nitrate, the synthesis efficiency of silver nanoparticles decreases. Also, increasing the concentration does not have much effect on the size of nanoparticles, since, in Figure 7 λ_{\max} , the different graphs are about 435 and the same, on the other hand, λ_{\max} is directly related to the size of nanoparticles. Therefore, increasing the nitrate concentration has little effect on the nanoparticle size

Investigation of the effect of temperature on the synthesis of silver nanoparticles:

The temperature has a great effect on the synthesis efficiency of nanoparticles. Synthesis of silver nanoparticles was performed at different temperatures (17, 35, 50, 70, and 120 degrees Celsius). As shown in Figure 8, with increasing temperature from 17 to 120 degrees Celsius, a significant increase in maximum absorption is observed. Performing the reaction at temperatures above 120 °C does not show a significant change in adsorption (the corresponding spectrum is not shown in the Figure).

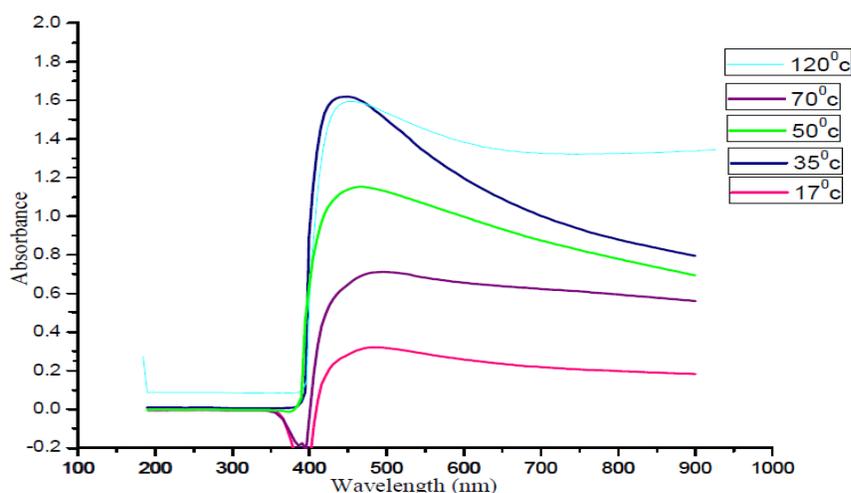


Figure 8. The effect of reaction temperature on the synthesis of silver nanoparticles

Therefore, 70 °C was chosen as the optimal temperature. Also, due to the direct relationship between the size of nanoparticles and the absorption wavelength, it seems that at temperatures of 17 to 50 °C, silver nanoparticles with different dimensions are formed, but at a temperature of 70 °C, the adsorption of the solution is in the range of 400 to 550 nm. With λ_{\max} is about 435, which indicates the synthesis of nanoparticles with

close dimensions so the temperature of 70°C was chosen as optimal [25].

Investigation of the effect of time on the synthesis of silver nanoparticles:

To achieve the best efficiency in the synthesis of silver nanoparticles, the effect of time on the above reaction was investigated. As shown in Figure 9, the time has a large effect on the synthesis efficiency of silver nanoparticles.

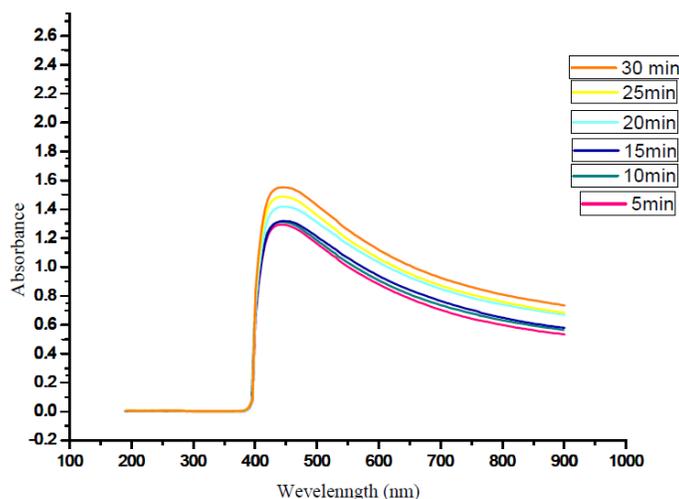


Figure 9. The effect of reaction time on the synthesis of silver nanoparticles

The synthesis of silver nanoparticles was studied during a period of 5 to 30 minutes and the results showed that the optimal time for the synthesis of nanoparticles is the first 5 minutes. Also, time does not seem to have a significant effect on the size of silver nanoparticles.

Investigation of the effect of pH on the synthesis of silver nanoparticles:

As shown in Figure 10, the most suitable pH for the synthesis of silver nanoparticles is 5.5, Which is the pH of the plant extract.

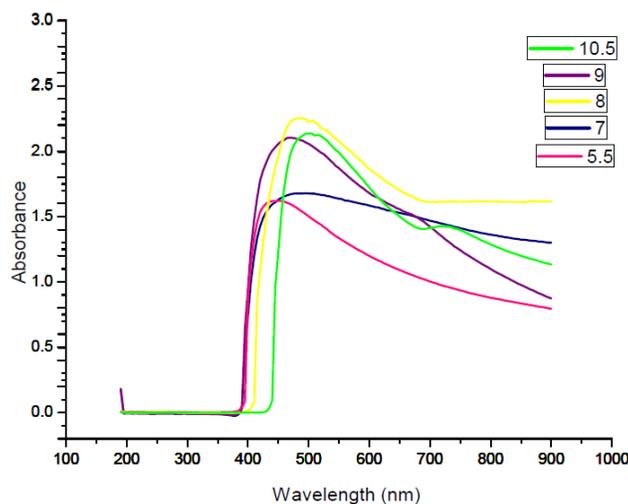


Figure 10. The effect of reaction PH on the synthesis of silver nanoparticles

Conclusion

Synthesis of *Stachys lavandulifolia* vahl plant extract can provide nanoparticles with

controlled size and shape. With the help of this plant extract, we propose a simple, economical, and effective way to synthesize silver nanoparticles and also show a green, fast, and

environmentally friendly chemistry approach for the synthesis of silver nanoparticles. Therefore, in this path, all conditions provide a 100% green chemical process. During this reaction, it was found that increasing the concentration of silver nitrate does not play a role in controlling the size of nanoparticles. Also, increasing the temperature was effective in the synthesis of silver nano, and 70 °C was selected as the optimal temperature. The time factor was not effective in the synthesis of this nano-silver and in the first 5 minutes the best production efficiency of nano-silver was obtained. Regarding the effect of pH parameters on the synthesis of nano-silver, it was observed that the highest efficiency is in plant pH. Antimicrobial silver using plant extracts showed that it can be used in various biomedical applications. Therefore, synthesized silver nanoparticles can have a high potential for use in biological applications.

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