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Optimization and characterization of biosynthesized silver nanoparticles by the extract of the thyme plant

Amin Baghizadeh^{a,*}, Fereshteh Fakhrabadipoor^b, Mohammadali Ebrahimi^b

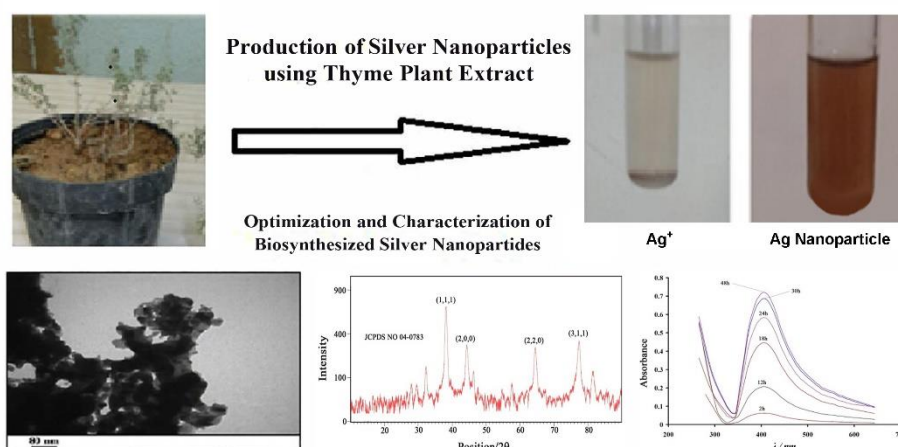
^a Department of Biotechnology, Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman, Iran

^b Department of Agricultural Biotechnology, Payame Noor University, Tehran, Iran

HIGHLIGHTS

- The simple process of reducing metal ions and producing nanoparticles is carried out by primary and secondary metabolites such as antioxidants, flavonoids, flavones, isoflavones, anthocyanidins, isothiocyanates, carotenoids, and polyphenols.
- The production of silver nanoparticles by thyme extract was confirmed by spectrophotometric analysis, TEM, XRD, and EIS.
- The maximum silver nanoparticle production by thyme extract was achieved within 30 h at 35 °C and pH=10.

GRAPHICAL ABSTRACT



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ABSTRACT

Nanotechnology is an attractive research field due to the production of nanoparticles in different sizes, shapes, chemical compositions, and distributions, and their many applications for humans. The fabrication, manipulation, and use of metallic nanoparticles are of great importance due to their reduced dimensions and, as a result, their unique thermal, optical, and electronic properties. Most nanoparticle synthesis techniques are inefficient considering material and energy consumption. In most chemical methods, dangerous chemical materials to humans and the environment are used. Accordingly, there is a demand for nanoparticle production using biological methods. Biological nanoparticle production methods have priority over physical and chemical methods considering energy cost, time reduction, and use of natural solvents. Currently, silver nanoparticles are one of the most commercial nanomaterials. In this research, silver nanoparticles were produced using thyme plant extract obtained using biological methods without any chemical materials. Various synthesis parameters such as temperature, pH, and synthesis time were optimized. The synthesized nanoparticles were characterized by various methods, including ultraviolet-visible spectrometry (UV-Vis), transmission electron microscopy (TEM), X-ray diffraction (XRD), and zeta potential. The synthesized nanoparticles are spherical, with a mean diameter of 38.9 ± 10.4 nm (range 20–60 nm) as measured by TEM. Results show that biosynthesis of silver nanoparticles using thyme extract is a successful, cost-effective, and time-efficient method.

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* Corresponding author: E-mail address: amin_4156@yahoo.com ; Tel: +98-9131414156

1. Introduction

Green synthesis of metal nanoparticles using plant extracts has emerged as a preferred alternative to physical and chemical methods, offering a low-cost, non-toxic, and environmentally friendly route that avoids hazardous reducing and stabilizing agents [1,2]. Compared with microbial systems, plant-mediated synthesis avoids the need for aseptic culture maintenance and is faster, cheaper, and more readily scaled up [3-6]. Among metal nanoparticles, silver nanoparticles (Ag NPs) are of particular commercial and biomedical interest owing to their well-documented antibacterial activity [2-4]. A range of plant extracts have been used for the phytosynthesis of silver and gold nanoparticles, including *Boerhavia elegans* [7], *Sansevieria* [8], Osage orange (*Maclura pomifera*) leaf [9], saffron petal [10] and *Moringa oleifera* [11], yielding nanoparticles that differ in size and morphology depending on the phytochemical profile of the extract and the reaction conditions used. These studies consistently show that reaction parameters — particularly pH, temperature, and reaction time — govern the reduction kinetics and the final size, shape, and stability of the nanoparticles, since they directly affect the activity of the phytochemicals (polyphenols, flavonoids, and terpenoids) responsible for reducing and capping the metal ions [12]. Thyme (*Thymus vulgaris*) is rich in terpenoids and phenolic compounds, particularly thymol and carvacrol, along with tannins, flavonoids, and saponins known for their strong reducing and antioxidant activity [13]. Despite its well-established antibacterial and antifungal properties and wide industrial use, thyme extract has been comparatively little explored as a reducing/capping agent for the biosynthesis of silver nanoparticles, and the effect of key synthesis parameters on this system has not been systematically optimized [14,15]. In the present study, silver nanoparticles were biosynthesized using thyme extract, and the effects of pH, temperature, and reaction time on nanoparticle formation were investigated and optimized; the resulting nanoparticles were characterized by UV-Vis spectroscopy, TEM, XRD, zeta potential, and electrochemical impedance spectroscopy (EIS).

2. Materials and methods

2.1. Preparation of silver nitrate solution

Silver nitrate (AgNO_3) was purchased from Merck (Germany), a 0.1 M stock solution was prepared in a volume of 50 mL, and this stock was used to prepare subsequent desired concentrations of silver nitrate. Next, to perform the

desired experiments, 3 mL of the extract was mixed with 47 mL of 1 mM silver nitrate solution.

2.2. Preparation of plant extract

Thyme seeds were prepared from Pakan Seed Company, Isfahan. The seeds were surface-disinfected with 2 % sodium hypochlorite for 10 min, then washed 3 times with sterile distilled water to remove sodium hypochlorite from the surface of the seeds. The seeds were placed on filter paper to dry. All these steps were performed in a laminar hood. Then, the disinfected seeds were sown in sterilized soil in pots. The pots were transferred to a greenhouse with appropriate light, temperature, and humidity conditions. After the seedlings reached the four-leaf stage, the seedlings were gently separated without damaging the roots and washed well with deionized water. In the next step, the hot soaking method was used for extraction. In this way, 50 g of fresh whole (root+ stem) thyme plants were added to 100 mL of boiling water after weighing. After 30 min, the extract obtained was passed through Whatman filter paper number 1. Fresh extract was used in all stages of the experiment. The whole seedling (root + stem) was used, rather than isolated leaves or aerial parts, in order to maximize the yield of extractable phytochemicals at the four-leaf stage and because the reducing potential of thyme root and stem tissue for silver biosynthesis has not previously been reported; comparison with leaf/aerial-only extracts is planned as a direction for future work.

2.3. Silver reduction by thyme plant extract

The color change of the extract was investigated after adding silver nitrate solution. Also, the absorption spectrum of thyme extract was analyzed by UV-Vis spectrophotometry (Cary50 / Conc) and again after 24 h to evaluate the biosynthesis of silver nanoparticles by the extract. Considering that silver has a maximum absorption peak in the range of 380-450 nm, wavelengths of 300 to 600 nm were considered for the biosynthesis of silver nanoparticles during the experiment. Distilled water was also used as a negative control.

2.4. Effect of time on the rate of extract absorption

In this experiment, to investigate the effect of time, silver nitrate solution was mixed with the prepared thyme extract, and spectroscopy was performed at 2, 12, 18, 24, 30, and 48 h, and the data obtained were studied to investigate the optimal time conditions.

2.5. Effect of temperature on the rate of extract absorption

At this stage, spectroscopy was performed to investigate the effect of temperature and determine the appropriate temperature within the optimized time of 30 h in the temperature range of 25, 35, and 50 °C, while the extract-to- AgNO_3 ratio and all other reaction conditions were kept constant at the levels used in the time-optimization step.

2.6. Effect of pH values on the rate of extract absorption

Next, the effect of different pH levels of 5, 8, and 10 was investigated under optimized time conditions of 30 h and at an optimized temperature of 35°C. Dilute sodium hydroxide and hydrochloric acid (0.1 M) were added to the plant extract to adjust its pH prior to mixing with the silver nitrate solution, thereby minimizing direct chemical interaction between these adjusting agents and AgNO_3 ; nonetheless, we acknowledge that a buffering system (e.g., citrate or phosphate buffer) would provide tighter control of ionic strength and will be adopted in future optimization studies. Temperature and reaction time were kept constant at their previously optimized values (35 °C and 30 h, respectively) throughout the pH experiments.

2.7. Characterization of nanoparticles

All UV-Vis measurements were performed in triplicate ($n = 3$) under each condition, and results are expressed as mean values. The synthesized sample at the optimal time, temperature, and pH was collected after centrifugation at 18,000 rpm for 20 min, and after drying, it was powdered and used for X-ray diffraction and transmission electron microscopy analyses. In addition, the zeta potential was also used to evaluate the nanoparticles. One of the important aspects of synthetic nanoparticles is their high ability to

increase electrical conductivity and reduce electron transfer resistance in electron exchange systems at the surface of a conductor. For this purpose, the electrochemical impedance method was used to prove the active presence of the produced silver nanoparticles and to investigate their effect on electrical signals.

3. Results and discussion

The synthesis of nanoparticles during the biosynthesis process was monitored using color change and UV-Vis absorption spectroscopy. Fig. 1 shows that before mixing thyme extract with silver nitrate, the absorption spectrum peak in the range of 380 to 450 nm was not observed. However, with the addition of the plant extract and the synthesis of silver nanoparticles, the absorption spectrum appears. This absorption spectrum has a maximum at about 420 nm [16]. On the other hand, as can be seen in Fig. 1, the silver nitrate solution is colorless, but after mixing with thyme plant extract, its color is yellow to brown, indicating the synthesis of silver nanoparticles [17]. This color change in most studies of biosynthesis of metal nanoparticles indicates surface plasmon resonance of metal [18,19].

Time is one of the most important factors in the formation of nanoparticles during biosynthesis techniques [20]. The oxidation-reduction process of the compounds in the extract can cause the reduction of silver nitrate and its conversion into silver nanoparticles. Therefore, time is important and necessary to complete this process and to completely reduce the silver nitrate and convert it into nanoparticles. A shorter time can weaken the possibility of first converting all silver ions and then converting them into nanoparticles; however, a longer time does not have a significant effect on this process. Therefore, to speed up the synthesis process and to completely synthesize nanoparticles, time optimization is important and necessary.

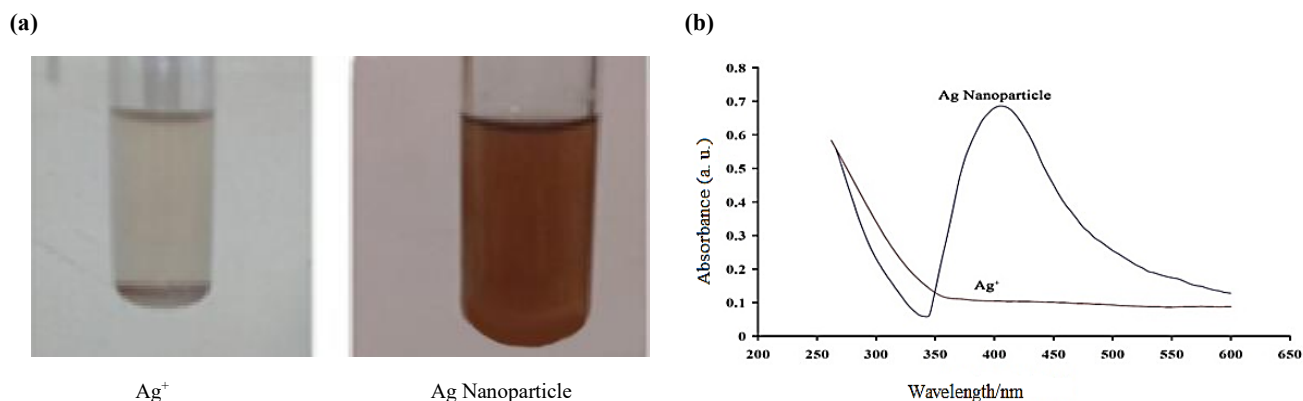


Fig. 1. Synthesis of silver nanoparticles by thyme plant extract. (a) Color change from colorless Ag^+ (left) to brown, indicating nanoparticle formation (right). (b) UV-Vis absorption spectrum of the reaction mixture, showing a peak near 420 nm characteristic of Ag NP surface plasmon resonance.

As seen in Fig. 2, as the time increases from 2 to 30 h, the silver adsorption rate increased, which is related to the increase in the concentration of synthetic silver nanoparticles. However, after that, within 48 h, the increase is almost constant, changing only slightly. This confirms that 30 h is sufficient to convert all silver ions into nanoparticles and that a longer time has no significant effect. In a study by Abu Torabi and colleagues, the optimal time for the synthesis of gold nanoparticles using barberry and saffron extracts was determined to be 24 h. They stated that this time is necessary for the reduction, nucleation, and formation of gold nanoparticles of the desired size and shape [21]. In another study investigating the synthesis of silver nanoparticles through the treatment of silver ions using the extract of the pepper plant, results showed that the crystalline phase of the nanoparticles changed from multicrystalline to single crystalline over time, and the size of the nanoparticles increased with increasing reaction time [22,23].

Although green synthesis of silver nanoparticles can proceed at room temperature, reaction temperature strongly influences the rate of reduction and the size of the resulting particles, since it affects the mobility and conformational stability of the phytochemicals responsible for silver-ion reduction. The effect of temperature was evaluated at 25, 35, and 50 °C at the previously optimized reaction time (30 h). As shown in Fig. 3, the UV-Vis absorbance increased when the temperature was raised from 25 to 35 °C, consistent with the higher kinetic energy and mobility of silver ions and reducing biomolecules at this temperature. However, at 50 °C the absorbance decreased markedly, most likely because the elevated temperature denatures or otherwise inactivates the proteins/enzymes and other reducing biomolecules in the extract, lowering their capacity to reduce Ag^+ to Ag^0 and to cap the resulting nanoparticles; excess thermal energy can also destabilize the colloidal suspension and promote aggregation and precipitation of larger particles. On this

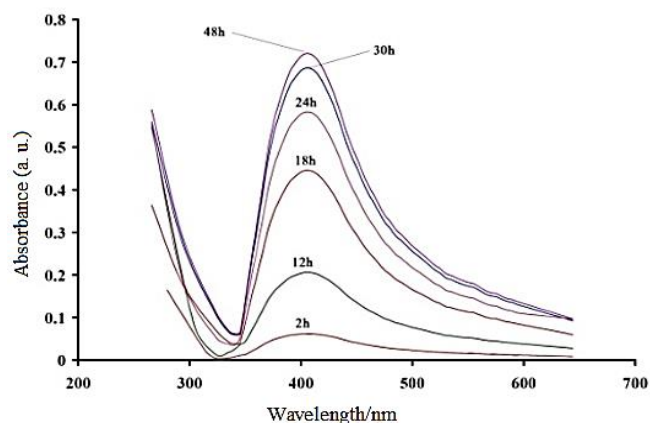


Fig. 2. UV-Vis spectrum of silver nanoparticles at different reaction times.

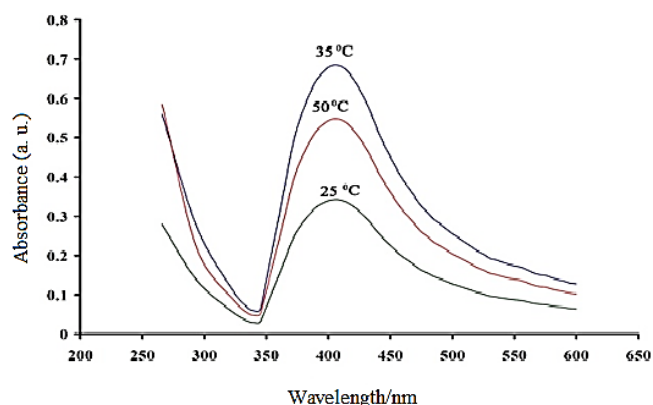


Fig. 3. UV-Vis spectrum of silver nanoparticles at different temperatures.

basis, 35 °C was selected as the optimal synthesis temperature for the thyme-extract system. The role of temperature in nanoparticle biosynthesis is system-dependent and varies with the type and thermal stability of the phytochemicals involved: in some plant systems elevated temperature accelerates reduction and favors uniform, smaller particles by increasing the frequency of nucleation events [24,25], whereas in extract systems containing more thermolabile reducing agents — as appears to be the case for thyme — excessive heat instead lowers synthesis efficiency once the effective operating temperature is exceeded. This interpretation is consistent with Soltani *et al.* [26], who likewise found 35 °C to be optimal and noted that higher temperatures increase molecular motion and promote nanoparticle interconnection/aggregation, supporting the view that the thyme-extract system behaves differently from systems in which higher temperature is uniformly beneficial.

The optimal activity of biomolecules is highly sensitive to the pH of the surrounding environment. It has been reported that the pH of the environment plays a very effective role in controlling the size of the particles. Larger nanoparticles are formed at lower pH. At acidic pH, the aggregation of larger nanoparticles around the core occurs to complete the new nanoparticle. At alkaline pH, the presence of a large number of functional groups for gold or silver bonding leads to the formation of a large number of nanoparticles with smaller diameters. At higher pH, the shape of most of the resulting nanoparticles is spherical and polyhedral, indicating the very important role of pH in controlling the shape and size of the synthesized nanoparticles [24-26]. Yuan *et al.* reported that with increasing pH, the reduction time of gold ions decreases, but the solution is unstable and precipitates after one hour [19]. Usually, with a relative increase in pH to 10, the size of the nanoparticles decreases. The effect of pH was investigated at three different pH values, including 5, 8, and 10 (Fig. 4). At basic pH, the presence of a large number of functional groups

for silver bonding leads to the formation of a large number of nanoparticles. Fig. 4 shows the results obtained from the effect of different pH values in the wavelength range of 300–600 nm. Based on the results obtained, pH 10 was determined as the optimum pH. At alkaline pH, the hydroxyl and carboxyl groups of the phenolic and flavonoid compounds in thyme extract (e.g., thymol, carvacrol, and tannins) become increasingly deprotonated, which enhances their electron-donating capacity and thus their efficiency as reducing and capping agents; this favors the formation of a larger number of nucleation sites and, consequently, a greater yield of smaller, more uniform nanoparticles, consistent with the results obtained at pH 10 in the present study.

The structure of biosynthesized silver nanoparticles under optimal environmental conditions was examined using transmission electron microscopy. Fig. 5(b) shows the synthesized silver nanoparticles. As can be seen, most of the synthesized silver nanoparticles have a spherical structure. Quantitative measurement of individual particles from the TEM image, calibrated against the scale bar, gave a mean diameter of 38.9 ± 10.4 nm (range 20–60 nm); the corresponding particle-size-distribution histogram is shown in Fig. 5(b). In a study, scanning electron microscopy (FE-SEM) imaging results of nanoparticles synthesized by extracts of barberry and saffron plants under optimal conditions confirmed that they were spherical and did not aggregate. In the biosynthesis of gold nanoparticles, plants are classified into three groups: those producing spherical nanoparticles in the size of 100–150 nm, triangular gold nanoparticles in the size of 50–100 nm, and gold nanoparticles in the size of less than 50 nm, which are used in pharmaceuticals [21]. El-Borady *et al.* investigated the green synthesis of gold nanoparticles using parsley leaf extract as a biocompatible method. Full characterization was done by TEM, SEM, XRD, FT-IR, UV-Vis, and zeta potential. Four samples with different shapes and sizes were obtained [27].

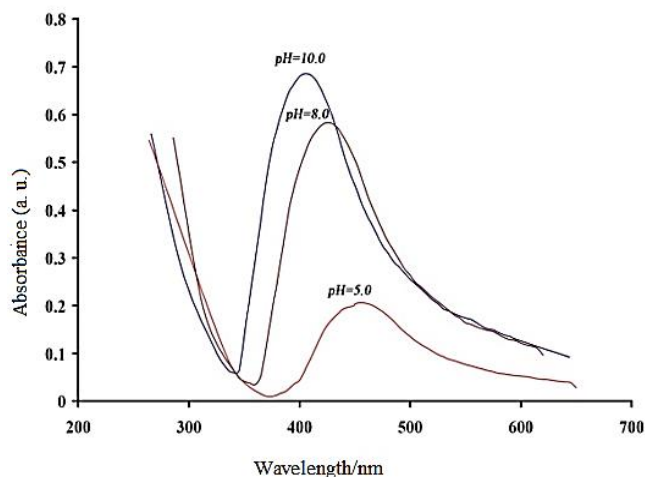


Fig. 4. UV-Vis spectrum of silver nanoparticles at different pH.

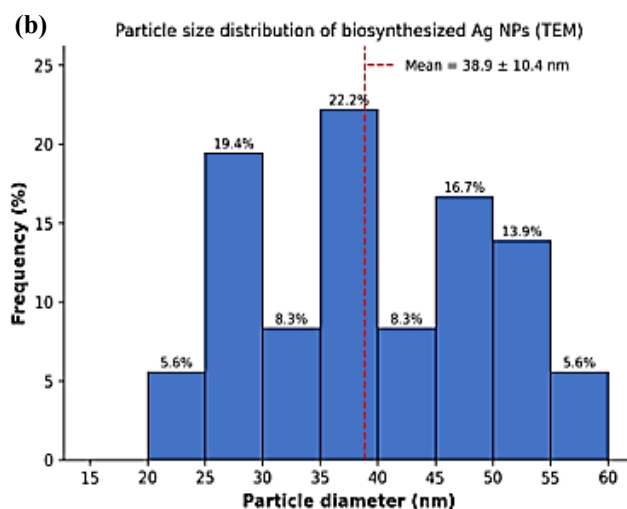
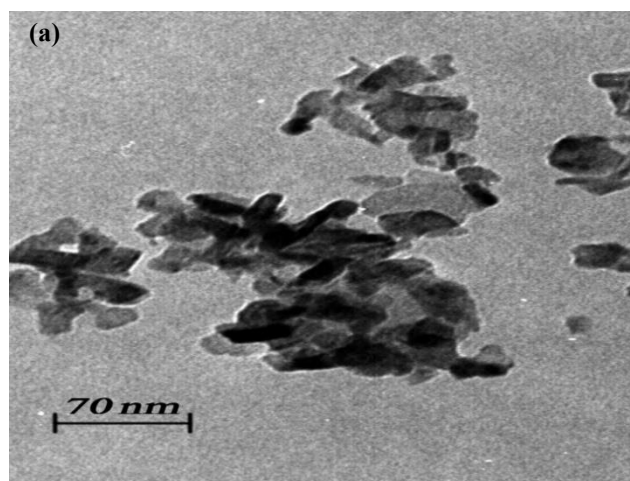


Fig. 5. (a) TEM of silver nanoparticles synthesized by thyme plant extract. (b) Particle size distribution histogram of biosynthesized Ag NPs.

According to Fig. 6, the silver nanoparticles had four peaks with Miller indices of (1 1 1), (2 0 0), (2 2 0), and (3 1 1) at positions of 2θ around 37° , 44° , 64° , and 77° . The presence of these peaks is consistent with the standard peaks of silver, and this spectrum proves the correctness of the synthesis of silver nanoparticles. In one study, leaf extract of *Ziziphus ziziphus* was investigated for the synthesis of gold nanoparticles [28]. The phytochemicals present in the aqueous leaf extract acted as an effective reducing agent. The AuNPs generated were characterized by TEM, SEM, atomic force microscopy (AFM), XRD, UV-Vis spectroscopy, and energy-dispersive X-ray spectroscopy (EDX). The prepared nanoparticles were found to be biocompatible. The XRD spectrum of the sample shows gold nanoparticles with a face-centered cubic crystal structure [28]. In a study conducted by Abu Torabi and colleagues on barberry and saffron plants, synthesized gold nanoparticles were characterized through UV-Vis spectrophotometry, TEM, and XRD. The XRD and TEM results confirmed AuNP production in

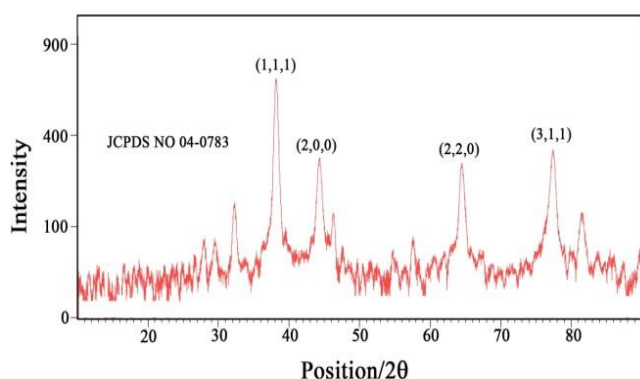


Fig. 6. XRD patterns of silver nanoparticles synthesized by thyme plant with silver nitrate solution.

5 - 15 nm for barberry and 5 - 10 nm for saffron and confirmed the stability, sphericity, and uniformity of both the nanoparticles [21].

To investigate the effect of nanoparticles on electrical signals, the electrochemical impedance method was used [29]. Fig. 7 shows (a) the Nyquist spectrum of 0.1 mM potassium ferricyanide (potassium hexacyanoferrate) on the electrode surface of plain carbon paste and (b) carbon paste modified with silver nanoparticles. In the presence of silver nanoparticles on the conductive surface, the radius of the semicircle corresponding to the impedance spectrum is reduced, which confirms the reduction in electron transfer resistance and increased conductivity of synthesized silver nanoparticles. In Ashraf et al., the firmness of the silver-based NPs was detected using the zeta potential and found to be -18.6 mV, and the value of the zeta potential was used to determine the solidity of the particles. A negative value clearly showed the suitability of the NPs and protected against their aggregation [30].

Gold nanorods synthesized by evening primrose seed extract (*Oenothera biennis*) had zeta potentials of -34.8 mV,

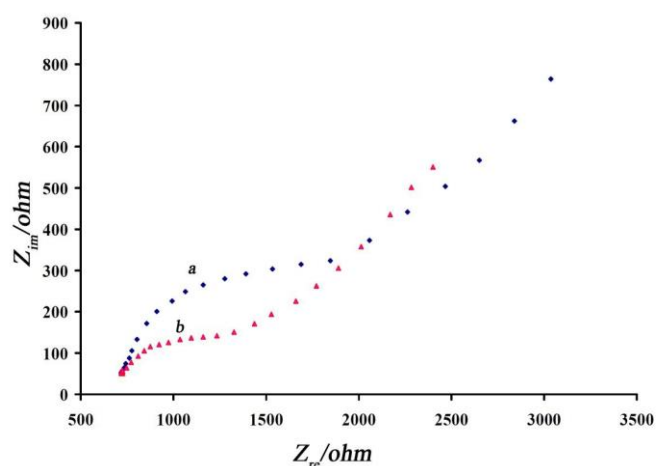


Fig. 7. Nyquist curve. (a) Simple carbon paste electrode. (b) Carbon paste electrode modified with silver nanoparticles.

and the resulting colloidal solution showed good stability or uniform distribution without aggregation and precipitation after six months [31].

4. Conclusion

This study demonstrates that thyme (*Thymus vulgaris*) extract, rich in phenolic and terpenoid compounds such as thymol and carvacrol, is an effective and previously underexplored reducing and capping agent for the green biosynthesis of silver nanoparticles. Reaction time, temperature, and pH were identified as key parameters governing nanoparticle formation, with maximal synthesis achieved at 30 h, 35 °C, and pH 10; deviations from these conditions reduced reduction efficiency, most likely due to thermal inactivation of the responsible biomolecules at higher temperatures and reduced deprotonation of functional groups at lower pH. UV-Vis, TEM, XRD, and electrochemical impedance analyses jointly confirmed the formation of spherical, crystalline silver nanoparticles with plasmon resonance near 420 nm and enhanced electron-transfer conductivity. Beyond establishing thyme as a viable green precursor, these findings support the wider case for plant-mediated nanoparticle synthesis as a scalable, low-cost, and environmentally benign alternative to chemical routes, with potential applications in antimicrobial coatings, biosensing, and conductive materials. Future work should include quantitative particle-size-distribution analysis, replicate-based statistical validation, and evaluation of the antibacterial and electrochemical performance of thyme-derived Ag NPs in applied settings.

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Additional information

Correspondence and requests for materials should be addressed to A. Baghizadeh.

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