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Evaluation of the Reducing Potential of *Abies nordmanniana* subsp. equi-trojeni Extract for Silver Particle Synthesis

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Abstract

This study evaluates the reducing potential of leaf extracts from the endemic species Abies nordmanniana subsp. equi-trojeni for the green synthesis of silver particles. The synthesis involved reacting a 1 mM silver nitrate ($AgNO_3$) solution with the plant extract at room temperature. We characterized both the reaction products and the supernatant using FTIR, UV-Vis, and SEM techniques. Our data confirm that phenolic compounds within the extract were actively consumed during the reduction of Ag^+ ions. Interestingly, SEM analysis revealed that the synthesized particles exhibited micrometer-scale dimensions rather than the expected nanoscale dispersion. We attribute this micro-structure formation to the high reducing power and concentration of the extract, which likely drove rapid nucleation followed by immediate agglomeration. These findings confirm the strong reductive capacity of Abies nordmanniana subsp. equi-trojeni and suggest that future protocol optimization—particularly regarding pH and concentration control—is necessary to fine-tune particle size from the micro- to the nanoscale.

Keywords: *Abies nordmanniana* Subsp. Equi-Trojeni; Green Synthesis; Silver Particles; SEM; FTIR; Reducing Potential

1. Introduction

While the capacity of plant extracts to reduce metal ions has been recognized since the early 20th century, the precise mechanisms of the natural reducing agents involved remain partially understood. Nevertheless, it is well-established that silver nanoparticles synthesized from phytochemical-rich extracts—containing compounds like quinones and proteins—tend to possess superior structural stability (Yavuz and Yılmaz, 2021). This advantage has led to a surge in materials science publications focusing on silver nanoparticles over the last decade (Badi'ah et al., 2019; Balasubramanian et al., 2019; Siddiqui et al., 2013).

Among the various methods for synthesizing silver nanoparticles, biological approaches have become increasingly prominent. Compared to physical and chemical techniques, biological methods are often preferred for their cost-effectiveness and environmental compatibility (Beykaya and Çağlar, 2016). Accordingly, the utilization of plant-based resources for silver nanoparticle synthesis has garnered significant attention, offering a simpler and more economical application process (Yuan et al., 2022).

For this study, we selected Abies nordmanniana subsp. equi-trojeni, an endemic species found in the Western and Central Black Sea regions. To date, there appear to be no studies in the literature utilizing this specific plant species for

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nanoparticle synthesis, although successful attempts using Picea abies (European Spruce) and other Abies taxa have been reported (Macovei et al., 2023; Macovei et al., 2022; Tanase et al., 2020; Wu et al., 2019).

While green synthesis research typically focuses on nanoparticle production, it is equally critical to understand how synthesis parameters—such as extract concentration—influence crystal growth and agglomeration. Identifying the conditions that shift products from the nanoscale to the microscale offers valuable insights into reaction kinetics and the extract's reducing power. Furthermore, the chemical composition of plants is known to vary with environmental factors, including climate, altitude, soil structure, and regional mineral balance.

2. Material and Methods

2.1. Plant Material

Abies nordmanniana subsp. equi-trojeni samples were collected between May and July from the Sarıkız vicinity of Kazdağı, Balıkesir (specifically, roadsides towards the summit).

2.2. Drying and Preparation

Freshly collected plant material was shade-dried at approximately 25°C in a low-humidity environment, avoiding direct sunlight. The dried samples were subsequently ground into a fine powder.

2.3. Preparation of the Plant Extract

We weighed 25 g of powdered plant material and heated it in 250 mL of purified water at 70-80°C. Once extract formation was observed, the mixture was allowed to cool to room temperature. After filtration, the extract was stored at 4°C for use in all experimental procedures.

2.4. Synthesis of Silver Particles

To synthesize silver particles, 125 mL of the plant extract was added to 500 mL of a 1 mM aqueous $AgNO_3$ solution. The mixture was left to react in a 1000 mL Erlenmeyer flask at room temperature. The resulting dark-colored solution was then centrifuged at 10,000 rpm for 5 minutes to separate the solid phase. The supernatant was removed, and the remaining solid product was washed multiple times with purified water. Finally, the product was oven-dried at 65°C for 48 hours prior to characterization.

2.5. Characterization

- FTIR Analysis: We performed IR analyses using a Perkin Elmer Spectrum One FTIR spectrometer (4000-600 cm^{-1}) to examine functional groups in the plant extract and identify changes in those groups post-reduction.
- UV-Vis Analysis: Electronic spectra of the reaction supernatant were recorded with a Perkin Elmer Lambda 25 UV-Vis spectrophotometer in the 200–900 nm range.
- SEM Analysis: Particle size and morphology were determined using a scanning electron microscope (SEM) EVO 40 LEQ.

3. Results

3.1. FTIR Spectroscopy Analysis

Comparison of the FTIR spectra for the Kazdağı Fir extract and the reaction supernatant (the liquid remaining after synthesis) reveals which functional groups participated in the reaction. The lack of distinct shifts in the 2800–1750 cm^{-1} range suggests these groups were not primary participants. However, shifts observed in the phenolic –OH peaks (3000-3600 cm^{-1}) indicate their involvement in silver particle formation. Specifically, the strong asymmetric stretching band of the –OH group in the extract shifted following the interaction with Ag^+ ions.

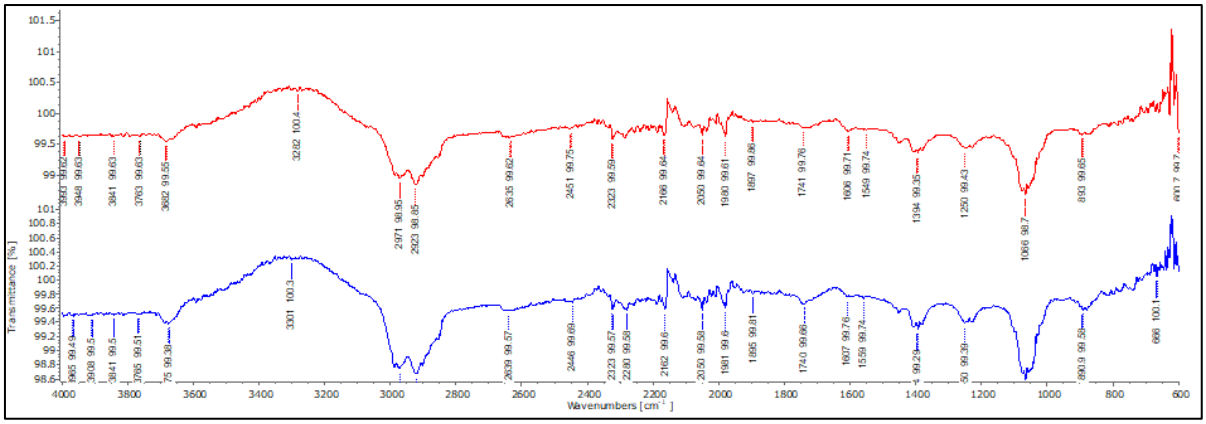

Figure 1 IR spectrum of plant extract (blue) and reaction supernatant (red)

3.2. UV-Vis Spectroscopy Analysis

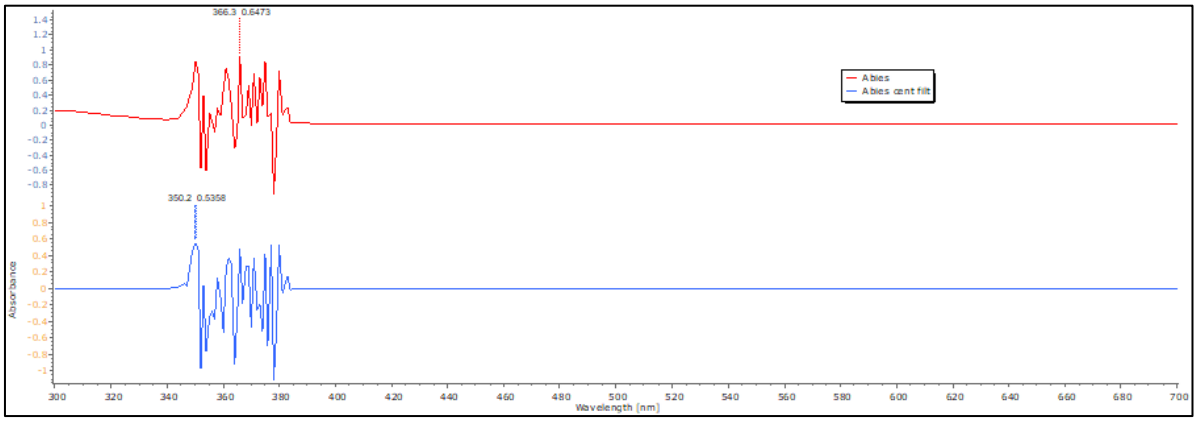

Figure 2 UV-Vi's spectrum of plant extract (red) and reaction supernatant (blue)

The Kazadi Fir extract exhibited a maximum absorbance wavelength of 366.33 nm (Absorbance: 0.64728, FWHM: 1.0141 nm). In contrast, the supernatant obtained after silver particle synthesis showed a maximum absorbance at 350.25 nm (Absorbance: 0.5358, FWHM: 3.1349 nm). This spectral change is significant, as it reflects the reduction of Ag^{+} ions by functional groups within the plant extract.

3.3. SEM Analysis

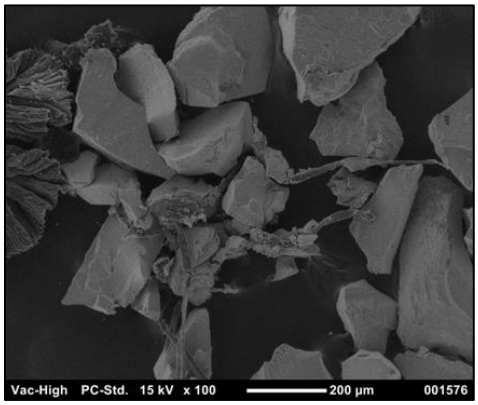

Figure 3 SEM image of silver particles

SEM analysis (EVO 40 LEQ) was used to determine the size and morphology of the synthesized silver particles. The images reveal that the particles are micrometer-sized rather than nano-sized. We suspect the high concentration of the extract played a key role in this outcome.

4. Discussion

This study reports the synthesis of silver particles using an aqueous extract of the endemic Abies nordmanniana subsp. equi-trojeni (Kazdağı Fir). To our knowledge, this is the first investigation into the reducing potential of this specific taxon for silver synthesis.

We monitored reaction progress via spectroscopic analysis of the supernatant. Significant changes in the FTIR spectra—particularly in the phenolic –OH stretching vibrations (3000-3600 cm^{-1})—confirm that polyphenolic compounds in the extract were actively consumed, acting as primary reducing agents for converting Ag^{+} to elemental silver (Ag^{0}). Additionally, UV-Vis analysis of the post-reaction supernatant showed a hypsochromic shift in maximum absorbance from 366 nm to 350 nm. This shift, combined with the absence of a distinct surface plasmon resonance (SPR) peak in the 400-450 nm range, indicates that the synthesized silver species did not remain as stable colloidal nanoparticles but instead precipitated out of the solution.

SEM analysis of the precipitate confirmed the presence of silver particles with irregular morphologies and micrometer-scale dimensions. While green synthesis often targets nanoscale dimensions, the formation of micro-sized particles here offers important insights into reaction kinetics. We attribute the observed particle growth to the balance between nucleation and growth rates. The high concentration of plant extract (1:4 ratio) likely provided an excess of reducing agents, triggering rapid nucleation. However, the subsequent capping mechanism may have been insufficient to prevent these nuclei from rapidly agglomerating—a process consistent with Ostwald ripening or secondary aggregation. The acidic nature of conifer extracts may have further facilitated the aggregation of nucleated particles into larger microstructures.

The formation of micrometer-scale structures observed in this study with *Abies nordmanniana* subsp. *equi-trojeni* aligns with our recent findings regarding other endemic species with high phenolic content. For instance, in a similar synthesis using *Sideritis trojana* Bornm., we reported that the high concentration of reducing agents triggered a 'burst' reduction of silver ions (Yardan, 2025b). This rapid reaction rate outpaced the stabilization process, resulting in uncontrolled nucleation and immediate aggregation, which mirrors the phenomena observed here.

Furthermore, our investigation into *Origanum sipyleum* L. extract also yielded silver microparticles, a result we attributed to mechanisms such as Ostwald ripening and a phytochemical 'bridging effect' caused by high extract saturation (Yardan, 2025a). These parallel findings across different endemic species strongly suggest that while these extracts possess robust reducing power, this potency can be counterproductive for nanoscale synthesis without strict kinetic control. Consequently, slowing the reaction velocity—potentially through dilution or pH modification—appears essential to favor discrete nanoparticle formation over micro-aggregates in such highly reductive systems.

5. Conclusion

In summary, our findings confirm that Abies nordmanniana subsp. equi-trojeni extract possesses a robust potential for reducing silver ions. Although the experimental conditions in this study favored the formation of micro-sized aggregates, these results establish a fundamental baseline for this endemic species. Future research should focus on optimizing parameters—specifically by reducing extract concentration, adjusting pH to alkaline levels, and exploring lower precursor concentrations—to achieve controlled dispersion and stability at the nanoscale.

Compliance with ethical standards

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