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(Research Article)

Green Synthesis of Silver Microparticles Using an Aqueous Extract of Endemic *Sideritis trojana* Bornm. and Their Characterization

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Abstract

The growing threat of antimicrobial resistance (AMR) demands novel therapeutic strategies. This research explores a plant-based 'green' synthesis route for producing silver particles, employing an aqueous extract from the endemic Turkish plant *Sideritis trojana* Bornm. The process aimed to provide a sustainable alternative to conventional chemical methods. The resulting particles were subjected to a suite of characterization techniques, including Fourier-Transform Infrared Spectroscopy (FTIR), UV-Vis Spectroscopy, and Scanning Electron Microscopy (SEM). Spectroscopic analysis by FTIR suggested the involvement of the extract's phenolic constituents in both the reduction of silver ions and the stabilization of the final product. While UV-Vis analysis confirmed a successful reduction reaction, microscopic evaluation via SEM unexpectedly revealed the formation of aggregated structures on the micrometer scale, not nanoparticles. This outcome is hypothesized to result from a highly concentrated extract, which likely induced rapid, uncontrolled particle growth and agglomeration. The study confirms the bioreductive potential of *S. trojana* extract but underscores the critical need for process optimization to control particle dimensions for potential future applications.

Keywords: Phytosynthesis; *Sideritis trojana*; Silver Microparticles; Green Chemistry; Characterization; Aggregation

1. Introduction

The efficacy of conventional antibiotics is diminishing globally, creating a formidable public health challenge known as antimicrobial resistance (AMR) (O'Neill, 2016). A landmark 2019 analysis quantified this threat, directly attributing 1.27 million deaths to bacterial AMR and associating it with nearly 5 million deaths overall, underscoring the pressing need for alternative antimicrobial solutions (Murray et al., 2022). Nanotechnology, particularly using silver nanoparticles (AgNPs), offers a compelling alternative due to its multifactorial mode of action against microbes (Wang et al., 2017).

Unlike single-target antibiotics, AgNPs can disrupt microbial integrity through several simultaneous pathways, such as cell wall destabilization, oxidative stress induction via reactive oxygen species (ROS), and intracellular damage following the release of silver ions (Ag^+) (Dakal et al., 2016; Hassan et al., 2022). This multi-target approach presents a significant barrier to the development of microbial resistance (Pal et al., 2007; Slavin et al., 2017).

While effective, conventional synthesis routes for AgNPs present significant drawbacks, including the use of toxic reagents, high energy requirements, and the production of hazardous waste (Iravani et al., 2014). Phytosynthesis, or the green synthesis of nanoparticles using plant extracts, has emerged as a scientifically robust and environmentally

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conscious alternative (Mittal et al., 2020). Plant extracts contain a rich cocktail of phytochemicals—such as polyphenols, flavonoids, and terpenoids—that act as natural bioreductants and capping agents, eliminating the need for external toxic chemicals (Makarov et al., 2014; Singh et al., 2018; Ghorbani et al., 2017).

The Lamiaceae family is particularly renowned for its wealth of medicinal plants whose extracts are rich in bioactive compounds (Hussain et al., 2018). The *Sideritis* genus ("mountain tea"), a member of this family, has a long-standing tradition in Mediterranean ethnopharmacology for treating inflammatory and infectious conditions (Tadic et al., 2021; González-Burgos et al., 2022). *Sideritis* species are generally known locally as "adacayi" or "dagcayi" and are widely used as herbal tea and folk medicine in Turkey (Paşa et al., 2019). The Lamiaceae family contains species of significant medicinal and economic value (Paşa, 2019); among these, the endemic Turkish species *Sideritis trojana* Bornm. is particularly rich in phenolic compounds possessing the redox properties necessary to drive metal ion reduction (Celep et al., 2019).Therefore, this research was designed to harness the bioreductive capacity of *S. trojana* aqueous extract for the synthesis of silver particles and to characterize the resulting material.

2. Materials and Methods

2.1. Plant Material and Extraction

Collected plant matter of Sideritis trojana Bornm. from Sarıkız, Kazdağı (Balıkesir, Turkey) was air-dried in the shade at ambient temperature (~25°C). The dried material was mechanically ground to a fine powder. For the extraction, 25 g of the powder was suspended in 250 mL of pure water and heated to 70-80°C to facilitate the release of phytochemicals. The resulting decoction was cooled to room temperature, filtered, and the aqueous extract was stored at 4°C prior to use.

2.2. Particle Synthesis

The synthesis was initiated by adding 125 mL of the prepared S. trojana extract to 500 mL of a 1 mM silver nitrate ($AgNO_3$) aqueous solution. The mixture was left undisturbed at room temperature to allow the reaction to proceed. The resulting dark precipitate was harvested by centrifugation at 10,000 rpm for 5 minutes. The collected solid was washed repeatedly with pure water to remove residual reactants and then dried in an oven at 65°C for 48 hours.

2.3. Characterization

The dried product was analyzed using three techniques. Functional group analysis was performed using a Perkin Elmer Spectrum One FTIR Spectrometer over a range of 4000-600 cm^{-1}. Optical properties were assessed with a Perkin Elmer Lambda 25 UV–Vis spectrophotometer scanning from 200 to 900 nm. Particle morphology and size were determined using an EVO 40 LEQ Scanning Electron Microscope (SEM).

3. Results and Discussion

3.1. FTIR Spectroscopy Analysis: Identifying the Bioreductive Agents

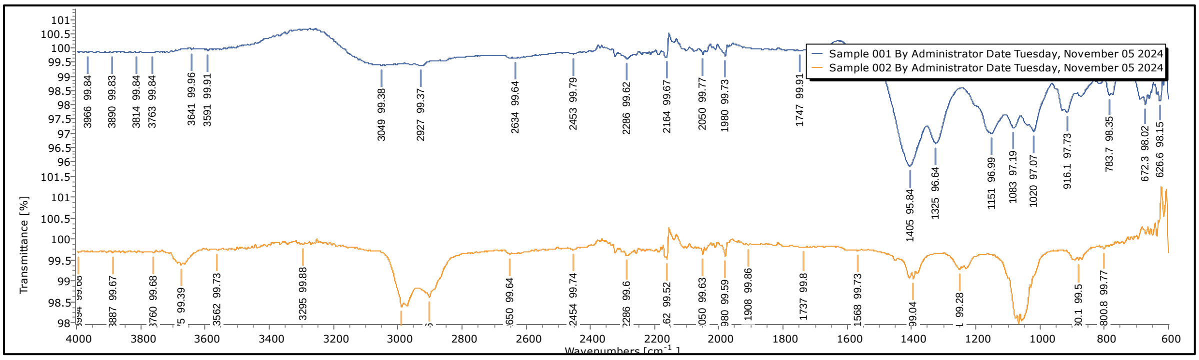

Figure 1 FTIR spectra of the Extract (1) and the Filtrate (2)

FTIR analysis was employed to identify the functional groups within the S. trojana extract that were actively involved in the synthesis and stabilization of the silver particles. The resulting spectra provided significant insights into the reaction mechanism. A prominent and broad absorption band observed between 3000-3600 cm^{-1}, characteristic of the

O-H stretching vibrations found in polyphenolic compounds, such as flavonoids and phenolic acids (Kharissova et al., 2019). The noticeable shift and broadening of this peak after the reaction strongly suggest that these phenolic hydroxyl groups were the primary participants in the reduction of Ag^{+} ions to metallic Ag^{0}. This finding is highly consistent with a large body of literature on phytosynthesis, where plant polyphenols are frequently identified as the key agents driving both the reduction and the subsequent capping of nanoparticles, preventing their immediate aggregation (Kharissova et al., 2019; Ghorbani et al., 2017). The data align perfectly with previous reports on the phenolic-rich composition of the Sideritis genus, confirming that these inherent phytochemicals are the engine of the synthesis process.

3.2. UV-Vis Spectroscopy Analysis: Monitoring the Reaction

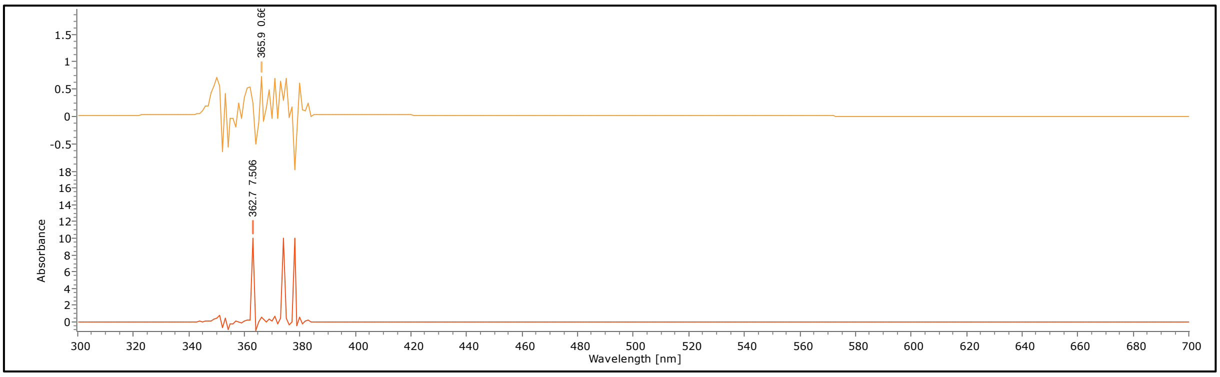

Figure 2 UV-Vis spectroscopy analysis of the Extract (top) and Filtrate (bottom)

The formation of silver particles was monitored using UV-Vis spectroscopy, a technique sensitive to the surface plasmon resonance (SPR) of metallic nanoparticles. Typically, spherical silver nanoparticles exhibit a distinct SPR peak between 400-450 nm (El-Nour et al., 2010). In our experiment, the aqueous extract initially showed a maximum absorbance at 365.93 nm. Following the reaction, the filtrate's maximum absorbance shifted to 362.75 nm. This spectral change, while not yielding a classic SPR peak in the expected range, confirms that a bioreduction reaction took place. The absence of a distinct peak around 420-440 nm is often indicative of either the formation of non-spherical particles or, more commonly, significant particle aggregation (Paramelle et al., 2014). When nanoparticles aggregate, the collective oscillation of electrons changes, leading to a dampening or shifting of the SPR peak, sometimes out of the characteristic range. This optical data provides the first piece of evidence that the reaction may have produced larger agglomerates rather than discrete nanoparticles.

3.3. SEM Analysis: Unveiling the Microparticle Morphology

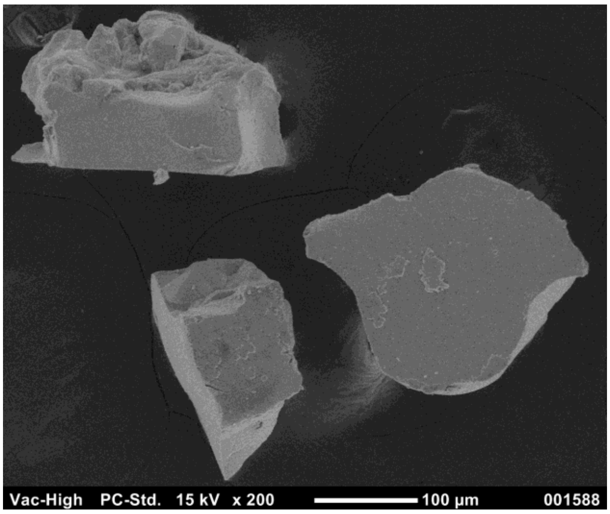

Figure 3 SEM image of silver particles

To obtain definitive information on particle size and morphology, SEM analysis was conducted. The resulting micrographs unequivocally showed that the synthesis did not yield nanoparticles (1-100 nm). Instead, the product consisted of large, irregularly shaped, and highly aggregated structures with dimensions firmly in the micrometer range.

This outcome, while not the initial objective, provides valuable insight into the kinetics of green synthesis. The literature presents conflicting reports on the effect of extract concentration; some studies report that higher concentrations lead to smaller particles due to an abundance of capping agents, while others, in line with our findings, report the formation of larger aggregates (Amin et al., 2021). The formation of microparticles in our study is strongly hypothesized to be a direct consequence of the high concentration of the *S. trojana* extract. A high concentration of potent reducing agents, such as the polyphenols in our extract, can lead to an extremely rapid, "burst" reduction of Ag^+ ions (Gudikandula & Maringanti, 2016). This creates a high density of initial nuclei that immediately and uncontrollably coalesce and aggregate before sufficient stabilization by capping agents can occur. This phenomenon, where the rate of reduction overwhelms the rate of stabilization, is a known challenge in phytosynthesis and directly leads to the formation of micro-scale structures instead of nano-scale ones (Jeevanandam et al., 2018). Our results are therefore a clear example of how critical the balance between the reducing and stabilizing capacity of a plant extract is, and how an overabundance of reducing power can be counterproductive to the goal of nanoparticle synthesis.

4. Conclusion

This study successfully demonstrated that the aqueous extract of *Sideritis trojana* possesses a high potential for the bioreduction of silver ions to metallic silver. However, the fact that the resulting particles were micrometer-sized, not nano-sized, reveals a critical mechanistic insight into the kinetics of green synthesis reactions. As detailed in the discussion, the high concentration of the extract led to an extremely rapid, 'burst' reduction that outpaced the stabilization process provided by the capping agents. This condition triggered uncontrolled nucleation and subsequent rapid agglomeration, resulting in the formation of microparticles.

This finding emphasizes that the mere presence of potent reducing agents in a plant extract is not sufficient for successful nanoparticle synthesis; the central challenge lies in managing the delicate balance between the rate of reduction and the rate of stabilization. Our results clearly show that an overly potent reductive environment can be counterproductive to achieving nano-scale dimensions.

Therefore, future work should focus on 'taming' this powerful reductive capacity. By systematically investigating the effects of modulating key parameters—such as lowering the extract-to-precursor ratio, adjusting the reaction temperature, and modifying the pH—the reaction kinetics can be slowed down to favor controlled, layer-by-layer particle growth over aggregation. Once this optimization is achieved, nanomaterials synthesized with *S. trojana* extract hold significant promise for valuable biomedical applications, particularly in contributing novel solutions to the ongoing fight against AMR.

Compliance with ethical standards

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