

Green Synthesis and Optimization of Monodisperse Silver Nanoparticles Using *Ocimum Sanctum* Aqueous Leaf Extract

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Abstract - This research article discusses the green synthesis of silver nanoparticles (AgNPs) with the help of aqagel leaf extract of *Ocimum sanctum* (Tulsi). The study aims to identify phytoconstituents of the *Ocimum sanctum* extract and their efficiency in forming noble metal nanoparticles. Qualitative phytochemical analysis has shown the presence of large amounts of phenols, reducing sugars, tannins, terpenoids, alkaloids, and glycosides. Also, it was noted that after 30 minutes of reaction time, the silver nitrate color changed from a colorless solution into a yellowish-brown color during the process of synthesis. Additionally, characterization was carried out by means of UV-Vis spectroscopy showing an SPR peak at the wavelength of 430 nm and HR SEM providing polymorphs of AgNPs with dimensions of 10–50 nm. The study has shown that this method is an environmentally friendly and inexpensive method for AgNPs synthesis.

Key Words: Silver nanoparticles, *Ocimum sanctum* (Tulsi) UV-Vis spectroscopy.

1. INTRODUCTION

Nanoscience and nanotechnology have surfaced as pivotal domains with revolutionary capabilities in healthcare, electronics, and agriculture. [1,2] The capacity to produce nanoparticles (NPs) with regulated dimensions, forms, and chemical compositions is essential for realizing their diverse applications. [3,4] Conventional chemical synthesis techniques, although efficient, frequently depend on hazardous reagents that present environmental and health hazards. Consequently, there is a growing emphasis on "green" chemistry and biosynthesis as sustainable alternatives. [5-7]

The synthesis of nanoparticles through plant mediation provides a dependable and environmentally sustainable method that circumvents hazardous chemicals while leveraging the biological attributes of plants, including their antimicrobial and anticancer properties. [8-11] Among the diverse flora examined, *Ocimum sanctum*, referred to as Tulsi or Holy Basil, is esteemed for its therapeutic properties. A constituent of the Lamiaceae family, it is abundant in bioactive phytochemicals. [12-15] It possesses an extensive historical application in Ayurvedic and Siddha medicine due to its adaptogenic, antimicrobial, cardio protective, and immunomodulatory attributes. Silver nanoparticles (AgNPs) are notably intriguing because of their distinctive electrical, catalytic, and antimicrobial characteristics. They are thoroughly investigated for their applications in electronics,

materials science, and medical treatments. [16-20] The application of *Ocimum sanctum* extract for the synthesis of AgNPs is beneficial due to its safety, accessibility, and the presence of metabolites that expedite the reduction of silver ions, consequently lowering production expenses and waste. [21-26] This research intends to harness the phytochemical abundance of *Ocimum sanctum* to create an eco-friendly approach for synthesizing AgNPs.

2. Materials and Methods

The methodology employed in this study encompasses the collection of plant material, extraction of phytochemicals, the synthesis of silver nanoparticles, and their subsequent characterization using spectroscopic and microscopic techniques

2.1 Plant Material Collection and Extraction

Fresh leaves of *Ocimum sanctum* were collected from the Kolli Hills, Tamil Nadu, India. Following authentication, the aqueous extract was prepared using a decoction method. Specifically, 10 grams of Tulasi leaves were boiled in 100 ml of distilled water for approximately 15 minutes. The boiling process continued until the solution attained a light yellowish hue. The resulting extract was then filtered and allowed to cool to room temperature before being used in subsequent analyses and synthesis.

2.2 Phytochemical Analysis

To determine the bioactive compounds responsible for the reduction and stabilization of silver ions, a qualitative phytochemical analysis was performed on 10 ml of the aqueous leaf extract. The tests included Phenols: Detected using the ferric chloride test, where a dark blue or greenish-black colour indicates presence.

Alkaloids: Confirmed using Mayer's test through the formation of a green or white precipitate.

Terpenoids: Identified via Salkowski's test, marked by a reddish-brown layer.

Flavonoids: Assessed using the sulphuric acid test for orange colour formation.

Tannins: Verified by the ferric chloride test.

Saponins: Determined by the persistent foam formation in the foam test.

Glycosides: Tested with sulphuric acid for a greenish-blue colour change.

Other Tests: Analysis also looked for quinones, carbohydrates (Molisch's test), proteins (Biuret test), triterpenoids, and anthraquinones.

2.3 Synthesis of Silver Nanoparticles (AgNPs)

The synthesis procedure involved mixing 95 ml of a 1mM silver nitrate (AgNO_3) solution with 20 ml of the prepared aqueous *Ocimum sanctum* extract. The reaction was monitored visually for colour changes, which occurred within 30 minutes as the clear solution turned yellowish-brown, signifying the reduction of silver ions to AgNPs. To purify the synthesized nanoparticles, the solution was centrifuged at 10,000 rpm for 10 minutes at 4 °C. The resulting pellets were collected and stored for further characterization.

2.4 Characterization Techniques

Two primary techniques were utilized to characterize the synthesized AgNPs:

1. UV-Visible Spectroscopy: This was used to confirm the formation of AgNPs by recording the absorption spectrum and identifying the characteristic Surface Plasmon Resonance (SPR) band.
2. Scanning Electron Microscopy (SEM): A Zeiss SEM machine was employed to analyse the morphology, size distribution, and aggregation state of the purified nanoparticles.

3. Results and Discussion

The experimental results confirm the successful extraction [Fig 1 & 2] of phytochemicals from *Ocimum sanctum* and their subsequent role in the formation of silver nanoparticles



Fig -1: : Tulsi leaves with solvent



Figure 2: Tulsi leaves extract

3.1 Phytochemical Screening

The qualitative screening of the aqueous leaf extract revealed a diverse array of phytochemicals. Phenol levels were very high, followed by moderate levels of alkaloids, glycosides, saponins, tannins, and terpenoids. Flavonoids, quinones, and triterpenoids were present at lower levels, while anthraquinones were notably absent. [Table -1] These phytochemicals, particularly the high phenolic content, are believed to serve as the primary agents for the reduction and stabilization of silver nanoparticles.

Phytochemical	Presence Status	Phytochemical	Presence Status
Phenols	Highly Present (+++)	Flavonoids	Present (+)
Alkaloids	Moderately Present (++)	Quinones	Present (+)
Glycosides	Moderately Present (++)	Triterpenoids	Present (+)
Saponins	Moderately Present (++)	Steroids	Absent (-)
Tannins	Moderately Present (++)	Proteins	Absent (-)
Terpenoids	Moderately Present (++)	Anthraquinones	Absent (-)

Table 1: Phytochemical Screening

3.2 Visual Observation and Synthesis

The reduction of silver ions into nanoparticles was marked by a distinct colour change in the reaction mixture. Within 30 minutes of adding *Ocimum sanctum* extract to the silver nitrate solution, the colour shifted from transparent to a characteristic yellowish-brown hue. This rapid change is indicative of the formation of metallic silver nanoparticles through the biological reduction process. [Fig 3-6]


Figure 3: AgNO₃ Solution

Figure 4: Tulsi Extract + AgNO₃


Figure 5: After centrifugation



Figure 6: Ag-Particles

3.3 UV-Visible Spectral Studies

Further evidence of AgNP formation was provided by spectroscopic analysis. In the UV-Visible spectrum, the characteristic SPR peak was observed at 430 nm. [Fig 7] The SPR peak is a fundamental property of metallic nanoparticles that arises from the collective oscillation of conduction electrons in response to incident light. The peak at 430 nm is consistent with the presence of spherical or near-spherical silver nanoparticles and confirms that the aqueous extract of *Ocimum sanctum* is an effective medium for synthesis.

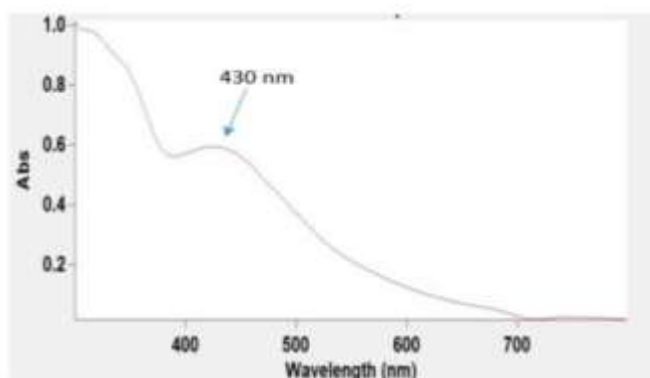


Figure 7: UV- Visible spectra

3.4 Morphological Analysis (SEM)

SEM imaging revealed that the synthesized AgNPs were polymorphic in nature. The nanoparticles exhibited various shapes, including spherical, ellipsoidal, rocky, flake-type, irregular granulated, compact, and fused forms. The size of these particles was measured to be between 10 and 50 nm. Importantly, the SEM images indicated that the nanoparticles were largely mono-dispersed, meaning they were relatively uniform in size distribution with minimal aggregation. [Fig 8] The lack of significant aggregation suggests that the phytochemicals in the Tulsi extract provided effective capping and stabilization.

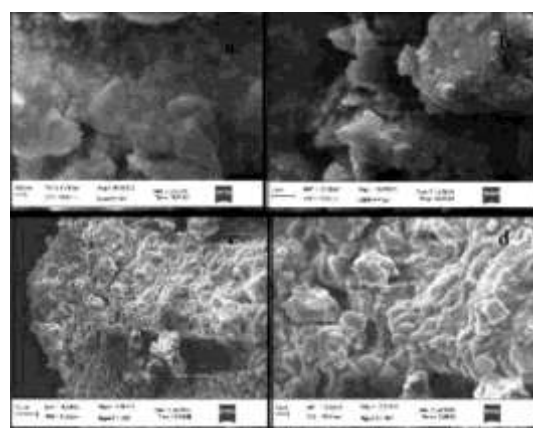
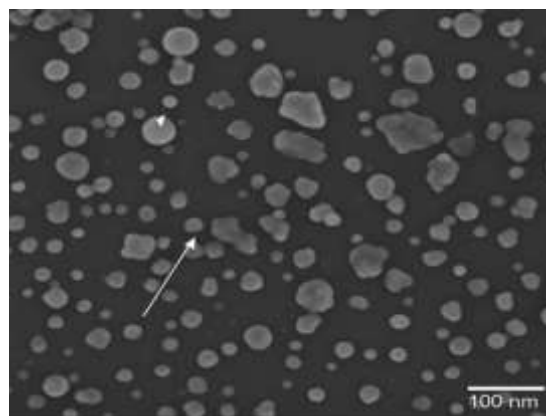


Figure 8: SEM images of (a & b). AgNPs

4. DISCUSSION

The change of color from light yellow to brown was first checked at 430 nm using UV visible spectroscopy. Obtaining plant extracts of *Ocimum sanctum* means using the strength of plants that possess various healing properties. Thus, one can say that creation of non-uniformly structured nanoparticles can help achieve this end. One of the main reasons why using plant techniques for production of nanoparticles is considered one of the best methods for this purpose is its eco-friendly nature and slow kinetics, which allow controlling the processes of formation of crystals and their stabilization. In addition, characterization was performed mainly using UV-Visible spectroscopy and SEM methods, whose usage provided some relevant information on the process of formation and structure of crystals. However, X-ray Diffraction XRD or Fourier-Transform Infrared Spectroscopy, for example, could contribute to better understanding of crystalline structure and the roles of functional groups in the process of capping. The success of this green synthesis approach highlights the efficiency of *Ocimum sanctum* as a natural bio-factory. The presence of diverse phytochemicals such as phenols and terpenoids facilitates a one-step synthesis process that eliminates the need for external chemical reducing agents. Compared to conventional methods, this process is not only more environmentally sustainable but also potentially more biocompatible for medical use. The variation in nanoparticle shapes (polymorphism) observed in SEM may be attributed to the complex mixture of phytochemicals involved in the reduction process.

5. CONCLUSIONS

The current study proposes a simple, inexpensive, and eco-friendly method of green synthesis of silver nanoparticles using the leaf extract of *Ocimum sanctum*. The presence of a multitude of chemicals in Tulsi, especially phenolic compounds, contributes to effective reduction of silver ions and stabilization of synthesized nanoparticles. The produced AgNPs were confirmed through visual inspection, distinct UV-Vis SPR peak, and SEM which demonstrated uniformity and polydispersity with sizes in the range of 10-50 nm. The results indicate the applicability of the synthesized *Ocimum sanctum* AgNPs in nanobiotech and pharmaceuticals thus paving the way for environmentally-friendly protocol in industrial-scale synthesis of nanoparticles. However, the studies on extraction, characterization and evaluation of toxicity of active components must be carried out. From the technology point of view, these silver nanoparticles have a significant number of applications in medicine.

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