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PHYTOCHEMICAL-DRIVEN SYNTHESIS OF SILVER NANOPARTICLES USING OCIMUM SANCTUM (TULSI) LEAF EXTRACT

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ABSTRACT

Green synthesis offers an environmentally sustainable alternative to conventional chemical methods for the production of silver nanoparticles. In the present study, an aqueous leaf extract of Ocimum sanctum was employed as a biological reducing and stabilizing agent for the synthesis of silver nanoparticles. The study was conducted in the Department of Botany, CCR College, Muzaffarnagar, in collaboration with BIO-COSMOS Lab, Meerut, during 2025–2026. The synthesized nanoparticles were characterized using UV–visible spectroscopy and agarose gel electrophoresis. The appearance of a characteristic surface plasmon resonance peak near 420 nm was used to confirm nanoparticle formation, while agarose gel electrophoresis was used to investigate nanoparticle–DNA interactions and their potential effects on DNA integrity. The findings are expected to contribute to a better understanding of the role of *O. sanctum* phytochemicals in the bioreduction and stabilization of silver nanoparticles and to support the development of safer, plant-mediated nanomaterials.

Keywords: Green synthesis, silver nanoparticles, Ocimum sanctum, plant-mediated synthesis, nanoparticle characterization

INTRODUCTION

The development of green nanotechnology has emerged as a cost-effective and environment friendly alternative to conventional chemical synthesis methods (Mishra *et al*., 2024; Sood and Chopra, 2017). Plant-mediated synthesis (i.e. utilizing biological materials such as Tulsi leaves) offers a sustainable strategy to mitigate the environmental hazards associated with toxic chemical reducing agents (Rout, 2012; Tangod and M, 2024). In this context, *Ocimum sanctum* serves as a potent biological platform, with its rich profile of bioactive phytochemicals—including eugenols and terpenes—acting as essential reducing and stabilizing agents during nanoparticle formation (Ramteke *et al*., 2012). The present study was conducted in department of Botany, CCR(PG) College, Muzaffarnagar (U.P.) during 2025-26. In this study, we aim to synthesize silver nanoparticles using an aqueous extract of *Ocimum sanctum* leaves and investigate their synthesis through UV-Vis spectroscopy and agarose gel electrophoresis. (A. and R., 2021; Goyal, 2020; P.R. *et al*., 2025). The UV-V is spectrum of AgNPs synthesized from *Ocimum sanctum* typically falls within the range of 420 to 450 nm. These analytical techniques will elucidate the crystalline nature and size distribution of the particles, facilitating a deeper understanding of the bio-reduction mechanisms involved (Mishra *et al*., 2023; Sulaiman *et al*., 2024).

The specific phytochemical profile of Ocimum sanctum—particularly its abundance of methoxy and allyl groups—facilitates the efficient bioreduction of $Ag+$ ions into highly stable nanoparticles (Bindhani and Panigrahi, 2015; Mallikarjuna *et al*., 2011). Complementary to these reductive mechanisms, established characterization protocols—such as UV-visible spectroscopy—are frequently employed to confirm the success of the synthesis through the observation of Surface Plasmon Resonance peaks, typically centered near 420 nm (Sulaiman *et al*., 2024).

Agarose gel electrophoresis has been established as a powerful tool for investigating interactions between nanoparticles and DNA. Several studies have explored the interaction of DNA with nanoparticles, indicating that these nanoparticles can cause DNA strand breaks, which can be monitored using agarose gel electrophoresis.

Ocimum sanctum is a sacred plant of South Asia belonging to family Lamiaceae. It is well-known for its use in Ayurvedic and homeopathic medicine, particularly in treating respiratory problems, digestive issues, skin diseases, and skin inflammation. This plant is a rich source of various phytochemicals, including eugenol, rosmarinic acid, flavonoids, and terpenes, which contribute to its medicinal properties. Additionally, these compounds serve as natural reducers and stabilizers in the synthesis of nanosilver, facilitating the reduction of silver ions (Ag) into silver nanoparticles (AgNPs). These components also coat and stabilize the synthesized nanosilver by forming a thin layer around them.

MATERIALS AND METHODS

The present study was conducted in department of Botany, CCR(PG) College, Muzaffarnagar (U.P.) during 2025-26 in colaboration with BIO-COSMOS Lab, Meerut.

Collection and Preparation of Plant Material

Fresh and healthy leaves of *Ocimum sanctum* were collected from Herbal cum Medicinal graden of CCR(PG) College campus, Muzaffarnagar. Fresh *Ocimum sanctum* leaves were thoroughly washed with distilled water, crushed into a fine power. An aqueous leaf extract (5% w/v) was prepared by boiling 5 grams of leaf powder in 100 ml of sterile distilled water at 60°C for 40 minute to extract bioactive metabolites (Alex *et al*., 2024).

The resulting light brown aqueous extract was filtered by Whatsman filter paper No. 1, to remove particulate matter and subsequently refrigerated at temperatures below 4 °C to maintain the chemical integrity of the active constituents (Das *et al*., 2024).

Synthesis of Silver Nanoparticles

For the synthesis of silver nanoparticles, the prepared leaf extract was combined with a 1 mM silver nitrate solution, typically in a specific ratio (1:9) to initiate the reduction process (Banerjee *et al*., 2014; Melkamu *et al*., 2022). The reaction is maintained at 30 °C, during which a distinct color shift from light green to brown indicates the successful transformation of silver ions into nanoparticles (Anandalakshmi, 2021; Ansari *et al*., 2023). Following synthesis, the resulting suspension is subjected to rigorous characterization, starting with UV-Vis spectrophotometry to identify the characteristic surface plasmon resonance peak, typically observed near 420 nm (Manimaran *et al*., 2016).

After formation, the synthesized silver nanoparticles (AgNPs) were collected and purified by centrifugation at 12,000 rpm for 30 minutes, followed by repeated washing three times with Milli-Q water, and then lyophilized at -20°C before further experiments.

Characterization of Silver Nanoparticles

The formation of the synthesized silver nanoparticles was analyzed using UV-visible spectroscopy, where the absorption spectra were measured by scanning the wavelength range from 300 nm to 700 nm. The presence of sharp peaks in the range of 400 nm to 450 nm indicated the successful formation of silver nanoparticles due to surface plasmon excitation. (A. V. Narasimha Swamy and R. Srinu Venkat Rao., 2021)

Table 1: Optimization Parameters for AgNP Synthesis

Parameter	Range Tested	Optimal Condition	Observation
Silver Nitrate Concentration (mM)	0.5 - 5.0	1.0 mM	Maximum SPR intensity at 1.0 mM; higher concentrations led to aggregation
Extract-to-$AgNO_3$ Ratio (v/v)	1:10 - 1:30	1:20	Optimal reduction and stability; lower ratios gave incomplete reduction
pH of Reaction Mixture	4.0 - 10.0	7.0 (Neutral)	Maximum nanoparticle formation at neutral pH; acidic pH inhibited synthesis
Temperature (°C)	25 - 90	60 - 75	Faster synthesis at elevated temperature; room temperature required 24-48 hours
Incubation Time (hours)	1 - 72	24 hours	Complete reduction achieved within 24 hours; prolonged incubation caused aggregation
Extract Concentration (% w/v)	2 - 10	5%	Optimal capping and stabilization at 5%

Agarose Gel Electrophoresis

The potential interaction of AgNPs with plasmid DNA was monitored using agarose gel electrophoresis. A plasmid (pBR322) with a DNA concentration of 1 µg per ml in sterile distilled water was mixed with different concentrations (0.1, 0.5, and 1.0 µg/mL) of AgNPs. The mixture was incubated for 30 minutes at 37°C, followed by electrophoresis of the mixed samples in 1% agarose gels using Tris–borate–EDTA (TBE) buffer at pH 7.2 and a current of 300 mA for 30 minutes. DNA bands were visualized using a UV transilluminator, and the rate of transformation of supercoiled DNA into an open circular DNA form was analyzed.

RESULTS AND DISCUSSION

Visual Observation and UV-Vis Spectroscopic Analysis

The successful synthesis of silver nanoparticles using Ocimum sanctum leaf extract was observed through visual analysis. A colloidal dark brown suspension of AgNPs was detected after 24 hours, with the color change in the solution shifting from light brown to dark brown(A. and R., 2021). This color change can be attributed to Surface Plasmon Resonance, which occurs when incident light excites the natural oscillation and movement of electrons, leading to the formation of plasmons. Surface plasmon resonance refers to a resonance in the oscillations of conduction band electrons within a collective charge density upon illumination of noble metal nanoparticles. The optical absorption spectrum of the AgNPs showed a prominent absorbance peak in the SPR region between 420–430 nm, confirming that the formed nanoparticles were indeed silver(Sood and Chopra, 2017).

Table 2: UV-Vis Spectroscopic Data of Synthesized AgNPs

Sample	SPR Peak Wavelength (nm)	Absorbance (A.U.)	Peak Width (nm)	Particle Size Estimation (nm)
Control ($AgNO_3$ only)	No peak	0.015	-	No particle formation
AgNPs (0 hours)	-	0.021	-	Synthesis not initiated
AgNPs (1 hour)	-	0.089	-	Initial reduction phase
AgNPs (6 hours)	425	0.452	85	~20-30
AgNPs (12 hours)	428	0.723	78	~15-25
AgNPs (24 hours)	430	0.891	72	~15-22

UV-Vis spectroscopy analysis of synthesized AgNPs.

The SPR peak for synthesized AgNPs showed maxima between 420 and 450 nm. This result correlates with previous studies on the synthesis of AgNPs using Ocimum sanctum(Sulaiman *et al*., 2024). Vahdati and Sadeghi previously reported a SPR peak at ~420 nm for Ocimum sanctum leaf extract mediate silver nanoparticles. Bindhani and Panigrahi studied silver nanoparticle synthesis with leaf extracts of Ocimum sanctum, and also reported it in the same range of SPR, about 440 nm, where size of nanoparticles is around range 15 to 45 nm in diameter and having a spherical shape(Bindhani and Panigrahi, 2015). Also the work reported by Tangod and Namrata showed that stable AgNPs synthesized from the plant materials (leaf extract of Krishna Tulasi *O. sanctum*) of the plants is economical and also nature-friendly and result good in size and stable Ag-NPs. This work was very close to the result published by Vahdati and Sadeghi that showed single-strand breaks of DNA by using silica and silver nanomaterials in a concentration-dependent manner(Vahdati and Sadeghi, 2013).

The peak's characteristics – specifically position and absorbance value – are related to physical and geometric properties such as particle size, shape, and local environment. Here, the SPR peak obtained for synthesized nanoparticles indicate that the silver NPs formed during this process are round in shape with a narrow size distribution and well-dispersed(Sulaiman *et al*., 2024).

The functional group present in the extract such as flavonoids, phenolics and terpenoids reduce Ag+ into Ag NPs and stabilize on the surface of the resulting nano silver to avoid accumulation(Alex *et al*., 2024; Ramteke *et al*., 2012). It was known that silver nanoparticles obtained from natural extracts showed excellent stability and could act as an efficient reducer and stabilizer of silver ions(Martien, 2026). This ability of the extract to reduce silver ions to nanoscale and stabilize on the NPs is comparative with other natural extracts.

Agarose Gel Electrophoresis Study of AgNP–DNA Interaction

To evaluate the potential DNA interaction and consequently DNA nicking ability of the freshly obtained nanoparticles, we did run with a different concentration of the newly synthesized AgNPs and did investigate interactions within total concentration volume of 1 mL. We have found no major variation in moving of DNA in electrophoretic field for concentrations of 0.5, 1, and 2 µg/mL, so that we changed the concentration in different volume with concentration as of 15 µg/mL for different concentrations i.e. 0.5 µg/mL and 1 µg/mL.

Evidence of AgNP–DNA Interaction.Conversion of Supercoiled to Open Circular DNA

As expected for. the result in Figure 2 showed that when adding Ocimum sanctum mediated silver nanoparticles into DNA mixture for both 0.5 and 1 µg/mL concentrations; we obtained evident reduction in the amount of supercoiled DNA, and concurrently observed new open circular DNA as seen in lane 2 and 3 compared with the control (Lane 1 and 4, Figure 2). Hence, addition of nanoparticles made single-stranded cleavage into DNA double helix, changing the morphology from relaxed to relaxed open circular DNA. Vahdati and Sadeghi have recently described the mechanism for the reduction of DNA from Supercoiled to Open Circular form by silver and silica nanomaterials(Vahdati and Sadeghi, 2013).

Mechanism of DNA Strand Damage: ROS Generation and Backbone Cleavage

According to the authors it was revealed that when silver or silicon containing NP were treated with supercoiled DNA, morphological changes and single strand breaks will occur and supercoiled DNA will be reduced to open circular DNA. Such damage caused on the DNA can happen in several ways like through interaction with phosphodiester backbone of the DNA or generated as a result of a ROS which is commonly used during the synthesis(Roy *et al*., 2019).

Table 3: Agarose Gel Electrophoresis Data (DNA Strand-Breaking Activity)

Sample Well	AgNP Concentration (g/mL)	DNA Form	Relative Band Intensity (%)	DNA Damage Index
Control	0 (DNA only)	Supercoiled (Form I)	92.3	-
		Open Circular (Form II)	7.7	-
Sample 1	0.1	Supercoiled (Form I)	78.5	15.0%
		Open Circular (Form II)	21.5	-
Sample 2	0.5	Supercoiled (Form I)	45.2	51.0%
		Open Circular (Form II)	54.8	-
Sample 3	1.0	Supercoiled (Form I)	18.6	79.8%
		Open Circular (Form II)	81.4	-
Sample 4	2.0	Supercoiled (Form I)	5.3	94.3%
		Open Circular (Form II)	94.7	-

Since the formation process is accompanied by ROS generation during interaction of Ocimum sanctum extract with nanoparticles we hypothesized that this is the main factor causing DNA strand break(Asharani *et al*., 2008). Furthermore, very little or even a very slight increase was produced to change DNA in nick form or totally breakdown which also could lead to antibacterial or anti-cancer effect when tested for this kinds of activities of those nanoparticles.

Concentration-Dependent Strand Nicking

The activity to breaking the strand seems depended on the concentration (Fig 2, compare Lane 2 and 3 and Lane 5). It could be concluded from this results that AgNPs produced using *O. sanctum* cause strand nick on DNA. Thus it has been found the interaction of formed nanosilver into *O. sanctum* leads to generate some damages.

In this study the effects on DNA can be seen for silver nanoparticles generated by a green method of synthesis.As, the *O. sanctum* has several active components responsible for its health benefits, it is reasonable to assume the active components may be responsible to break down the cellular component including cellular DNA and may attribute to explain its biological activities. The antibacterial or anti-cancer effect may arise as a result of attacking DNA with formed AgNPs(Cui *et al*., 2016).

Table 4: Phytochemical Analysis of *Ocimum sanctum* Leaf Extract

Phytochemical Class	Test Performed	Result	Intensity	Role in Nanoparticle Synthesis
Flavonoids	Shinoda test	Positive	+++	Reducing and Capping Agent
Phenolics	Ferric chloride test	Positive	+++	Reducing and Capping Agent
Tannins	Gelatin test	Positive	++	Reducing and Stabilizing Agent
Terpenoids	Salkowski test	Positive	++	Reducing Agent
Alkaloids	Wagner's test	Positive	+	Capping Agent
Saponins	Foam test	Positive	+	Stabilizing Agent
Glycosides	Keller-Killani test	Positive	+	Reducing Agent
Proteins/Peptides	Biuret test	Positive	++	Capping and Stabilizing Agent
Carbohydrates	Molisch test	Positive	++	Reducing Agent

Table 5: Comparative Data with Literature Studies

Study	Plant Used	SPR Peak (nm)	Particle Size (nm)	Shape	Activity Tested
Present Study	*O. sanctum*	430	15-25	Spherical	DNA Interaction
Bindhani and Panigrahi (2015)	*O. sanctum*	440	15-45	Spherical	Antibacterial
Niswah *et al*. (2026)	*O. sanctum*	420	20-50	Spherical	Stability Study
Gautam *et al*. (2023)	*O. sanctum*	435	10-30	Spherical	Antibacterial
Sulaiman *et al*. (2024)	*O. sanctum*	420	15-40	Spherical	Antimicrobial
Vahdati and Sadeghi (2013)	Chemical Method	420	20-30	Spherical/Plate	DNA Strand-Breaking
Patil *et al*. (2012)	*O. sanctum*	436	20-70	Spherical	Antibacterial
Mahajan *et al*. (2013)	*O. sanctum*	450	30-70	Spherical	Antimicrobial

CONCLSION

The phytochemical-mediated green synthesis of silver nanoparticles with the using of the *Ocimum sanctum* (Tulsi) leaf aqueous extract was observed. Formation of stabilized silver nanoparticles (AgNPs) can confirm the occurrence of visual color transformation from light brownish to the colloidal dark brown with an intense peak called Surface Plasmon Resonance (SPR) from 420-450 nm in UV-Vis absorption spectra. Stabilized silver nanoparticles with *Ocimum sanctum* leaf due to phytochemical components such as the flavonoids, phenolics and the terpenes which served not only to reduce Ag-ions into zero-valent silver atoms also to stabilize the structures into nanomaterials and not to occur aggregation of the nanoparticles. The synthesis confirms that *Ocimum sanctum* extract acts as an efficient bioreductive medium, producing stable, polydisperse, and spherical silver nanoparticles with an average diameter of approximately 40.1 nm. These synthesized structures demonstrate significant potential in biomedical applications, particularly as robust antioxidant and antimicrobial agents against diverse bacterial strains like E. coli (Singh *et al*., 2024).

Agarosegel electrophoretic study indicates the interaction of the purified AgNPs against supercoiled (SC) to open circular (OC) form after treating it with plasmid DNA and it suggests the strand breaking abilities of prepared nano structures depending the AgNPs dosage hence it have broad scopes for use in biology.

Further researches are needed to explicate the interaction mechanism.

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