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Green Nanotechnology Approach for Synthesizing Silver Nanoparticles from Ocimum sanctum: Characterization and Antimicrobial Evaluation Against Common Waterborne Bacteria

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Abstract

The present study explores an eco-friendly approach to synthesizing silver nanoparticles (AgNPs) using aqueous leaf extract of Ocimum sanctum (holy basil) and evaluates their antibacterial efficacy against common waterborne pathogens. The biosynthesis method employed the reducing and stabilizing capabilities of phytochemicals naturally present in the extract, eliminating the need for hazardous chemicals. Visual observation confirmed the formation of nanoparticles through a color change, while UV-Vis spectroscopy indicated surface plasmon resonance peaks between 423–438 nm. Characterization techniques, including FTIR, XRD, and TEM, revealed the involvement of hydroxyl and carbonyl groups in nanoparticle formation, confirmed their crystalline nature, and showed predominantly spherical particles ranging from 12–30 nm. Antibacterial activity was assessed via the agar well diffusion method against Escherichia coli, Salmonella typhi, and Vibrio cholerae. All AgNP formulations exhibited significant antibacterial activity, with formulation F5 showing the highest zones of inhibition, suggesting increased efficacy with higher extract concentration. The study demonstrates the potential of O. sanctum-mediated AgNPs as effective, sustainable antimicrobial agents for combating microbial contamination in water. This green nanotechnology approach offers a promising solution for water disinfection, especially in low-resource and environmentally sensitive settings.

Keywords: Green synthesis, silver nanoparticles (AgNPs), Ocimum sanctum, Waterborne pathogens, Antibacterial activity, Nanotechnology in water purification, Eco-friendly nanomaterials, Environmental nanobiotechnology

INTRODUCTION

Water is a fundamental resource for life, yet access to clean and safe drinking water remains a challenge for millions around the world. Contaminated water sources are major contributors to the global burden of disease, particularly in low- and middle-income countries where water treatment infrastructure is inadequate or poorly maintained. The presence of pathogenic microorganisms such as *Escherichia coli*, *Salmonella typhi*, and *Vibrio cholerae* in water bodies poses a serious public health threat, leading to outbreaks of waterborne diseases such as diarrhea, typhoid, and cholera. According to reports from the World Health Organization (WHO), waterborne illnesses result in the death of more than 3.4 million people each year, the majority of whom are children under five years of age. Hence, addressing microbial

contamination of water sources is an urgent public health priority (Madikizela & Pakade, 2023; Saravanan *et al.*, 2023; Xia *et al.*, 2023).

Traditional water purification methods, including chlorination, ozonation, and ultraviolet (UV) disinfection, have been employed with varying degrees of success. While these approaches are effective, they are often associated with limitations. For example, chemical disinfectants like chlorine can produce harmful disinfection by-products (DBPs), and some pathogens have developed resistance to conventional treatment methods. Moreover, these techniques often require significant infrastructure, technical expertise, and financial investment, which may not be feasible in under-resourced or rural areas. Consequently, there is a growing interest in developing alternative disinfection strategies that are both effective and environmentally sustainable (Jiang *et al.*, 2024; Lam *et al.*, 2023; Nan *et al.*, 2023).

Nanotechnology has emerged as a transformative field with vast potential for solving challenges in medicine, agriculture, energy, and environmental science. In the context of water purification, nanomaterials, particularly metallic nanoparticles, have gained attention due to their unique physicochemical properties. Among these, silver nanoparticles (AgNPs) have been extensively studied for their potent and broad-spectrum antimicrobial activity. AgNPs can interact with microbial membranes, disrupt cell wall integrity, produce reactive oxygen species (ROS), and interfere with microbial DNA replication and protein function, ultimately leading to cell death. These multifaceted mechanisms make silver nanoparticles particularly effective against a wide range of pathogenic bacteria, fungi, and viruses (Gahlawat *et al.*, 2016; Swamy *et al.*, 2015; Tamboli & Lee, 2013).

Despite their promising applications, conventional methods for synthesizing silver nanoparticles typically involve the use of toxic reducing agents (e.g., sodium borohydride, hydrazine), high temperatures, and expensive equipment, which pose environmental and safety concerns. To overcome these drawbacks, green synthesis methods have been developed using natural products such as plant extracts, microorganisms, and biopolymers as reducing and stabilizing agents. Green synthesis offers several advantages, including environmental safety, economic feasibility, ease of scaling up, and biocompatibility of the end product.Among various biological sources, plant-based synthesis of nanoparticles has received considerable attention due to the abundance of phytochemicals in plants that can facilitate reduction and stabilization processes. Plant-mediated synthesis does not require aseptic conditions and is generally faster and more efficient compared to microbial synthesis. Extracts from leaves, stems, roots, flowers, and seeds have been used successfully for the synthesis of various metal nanoparticles. The rich variety of secondary metabolites such as flavonoids, phenolics, terpenoids, alkaloids, and proteins present in plant extracts play a vital role in the bio-reduction of metal ions to their corresponding nanoparticles (Islam *et al.*, 2021; Kumari *et al.*, 2023).*Ocimum sanctum*, commonly known as Tulsi or holy basil, is a revered medicinal plant in traditional Indian systems of medicine such as Ayurveda and Siddha. It belongs to the Lamiaceae family and is widely distributed across the Indian subcontinent and Southeast Asia. The plant has been used for centuries for its therapeutic properties, including antimicrobial, anti-inflammatory, antioxidant, antipyretic, and adaptogenic effects. Its bioactive compounds such as eugenol, ursolic acid, rosmarinic acid, apigenin, and various flavonoids contribute to its pharmacological actions. Previous studies have confirmed the antimicrobial potential of *O. sanctum* against a wide range of Gram-positive and Gram-negative bacteria (Cohen, 2014; Khatoon *et al.*, 2022; Kumari *et al.*, 2024). Given its rich phytochemical profile and known antimicrobial properties, *O. sanctum* is an ideal candidate for the green synthesis of silver nanoparticles. The phenolic and flavonoid constituents present in the plant extract can effectively reduce silver ions (Ag^{+}) to metallic silver (Ag^{0}), while other biomolecules serve as capping agents to stabilize the nanoparticles and prevent agglomeration. The dual functionality of *O. sanctum*—as both a reducing and stabilizing agent and as a source of antimicrobial compounds—enhances the effectiveness of the synthesized AgNPs (Khatoon *et al.*, 2022; Kumar & Patel, 2023; R *et al.*, 2022).

The present study focuses on the green synthesis of silver nanoparticles using aqueous leaf extract of *Ocimum sanctum* and evaluates their antibacterial activity against selected waterborne pathogens. The synthesis process was optimized by varying the volume of plant extract used, and the resultant nanoparticles were characterized using multiple techniques. UV–Visible spectroscopy was employed to confirm the formation of AgNPs through the detection of characteristic surface plasmon resonance (SPR) peaks. Fourier Transform Infrared Spectroscopy (FTIR) was used to identify the functional groups

responsible for reduction and stabilization. The crystalline nature of the nanoparticles was determined by X-ray Diffraction (XRD) analysis, and their size and morphology were assessed using Transmission Electron Microscopy (TEM). Furthermore, the antibacterial efficacy of the synthesized nanoparticles was tested using the agar well diffusion method against *E. coli*, *S. typhi*, and *V. cholerae*, which are commonly implicated in waterborne infections. The influence of different extract volumes on particle size, shape, and antimicrobial activity was systematically studied to identify the most effective formulation.

This investigation not only underscores the utility of *O. sanctum* in sustainable nanomaterials synthesis but also demonstrates the potential of integrating traditional medicinal knowledge with modern nanotechnology for addressing contemporary public health issues. The eco-friendly approach adopted in this study supports the broader goal of sustainable development and offers a scalable and cost-effective solution for microbial decontamination of water. Importantly, the use of a readily available medicinal plant makes this technology accessible to rural and economically challenged communities where commercial water purification systems may be unaffordable or impractical. In conclusion, the current research aims to bridge the gap between traditional plant-based remedies and cutting-edge nanoscience by utilizing *Ocimum sanctum* for the biosynthesis of silver nanoparticles with targeted antibacterial applications. The findings are expected to contribute to the development of innovative, low-cost, and eco-conscious strategies for improving water quality and preventing infectious diseases caused by waterborne pathogens. This investigation not only highlights the importance of green nanotechnology in combating microbial contamination but also promotes the use of medicinal plants in developing sustainable water treatment solutions. The findings of this study could pave the way for the development of low-cost, plant-based nanomaterials for environmental and public health applications, especially in underserved rural and peri-urban communities.

2.MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Material

Fresh and healthy leaves of *Ocimum sanctum* were collected from a local herbal garden. The leaves were thoroughly washed under running tap water to remove dust and debris, followed by rinsing with distilled water. The cleaned leaves were shade-dried for 5–7 days at room temperature and then ground into a fine powder using a mechanical grinder. The powdered material was stored in an airtight container until further use.

2.2 Preparation of Aqueous Leaf Extract

To prepare the extract, 10 g of dried leaf powder was mixed with 100 mL of distilled water in a conical flask and boiled at 60–70°C for 30 minutes with continuous stirring. The mixture was allowed to cool, then filtered through Whatman No. 1 filter paper. The resulting clear filtrate was stored at 4°C for use in nanoparticle synthesis.

2.3. Biosynthesis of Silver Nanoparticles

For the green synthesis of silver nanoparticles (AgNPs), a 1 millimolar (1 mM) aqueous solution of silver nitrate ($AgNO_3$) was freshly prepared using analytical-grade silver nitrate (Sigma-Aldrich, USA). The solution was prepared in deionized water and stored in amber-colored glassware to protect it from light-induced degradation. In a typical synthesis procedure, 10 mL of the aqueous leaf extract of *Ocimum sanctum* was added dropwise to 90 mL of the 1 mM $AgNO_3$ solution under continuous magnetic stirring (Khatami *et al.*, 2017). The addition was carried out slowly over 10–15 minutes to ensure uniform mixing and effective interaction between the silver ions and the phytoconstituents present in the extract. The entire reaction was conducted at room temperature (approximately 25–27°C) and in a dark environment to prevent photoreduction of silver ions, which can otherwise affect the rate of nanoparticle formation and their size distribution. The reaction mixture was maintained under constant stirring for an additional 2 hours post-addition, followed by incubation in the dark at room temperature for 24 hours. A gradual change in the color of the solution from pale yellow to varying shades of brown served as a visual indicator of nanoparticle formation. This colour change is attributed to the excitation of surface plasmon vibrations of silver nanoparticles, confirming the successful reduction of Ag^+ to Ag^0 by the phytochemicals present in the *O. sanctum* extract. The intensity of the brown colour increased with time, indicating progressive formation and stabilization of silver nanoparticles. Different volumes of *O. sanctum* extract (5 mL to 25

mL) were used in separate batches to evaluate the effect of extract concentration on nanoparticle size, morphology, and antibacterial activity. These formulations were labelled F1 through F5, with increasing extract content. The synthesized nanoparticles were further subjected to purification by centrifugation at 12,000 rpm for 15 minutes, washed thrice with distilled water to remove unbound plant metabolites, and then re-dispersed in deionized water for subsequent characterization and biological testing. This simple, one-step biosynthetic approach leverages the reducing and capping capabilities of *O. sanctum*'s phytoconstituents to generate stable, bio-functional silver nanoparticles without the need for hazardous chemicals or high energy input, thus aligning with principles of green chemistry (Khatami *et al.*, 2017; Küp *et al.*, 2020).

Table 1: Composition of Green Synthesized Silver Nanoparticle Formulations Using *Ocimum sanctum*

Formulation Code	Volume of *O. sanctum* Extract (mL)	Volume of 1 mM $AgNO_3$ Solution (mL)	Final Volume (mL)	Color Change Observation
F1	5	95	100	Light brown
F2	10	90	100	Brown
F3	15	85	100	Dark brown
F4	20	80	100	Intense brown
F5	25	75	100	Very dark brown

2.4 Characterization of Synthesized Silver Nanoparticles

The successful formation and physicochemical properties of the silver nanoparticles (AgNPs) synthesized using *Ocimum sanctum* leaf extract were confirmed through a combination of analytical techniques.

UV–Visible Spectroscopy

The initial confirmation of nanoparticle synthesis was conducted using UV–Vis spectroscopy (Shimadzu UV-1800). The reaction mixtures were scanned in the range of 300 to 600 nm. The appearance of a characteristic surface plasmon resonance (SPR) peak between 420–440 nm indicated the formation of silver nanoparticles. The shift in SPR peaks among different formulations (F1 to F5) also provided insight into particle size variations and surface properties. A red shift in the absorption maxima with increasing plant extract concentration suggested smaller particle sizes and enhanced surface stabilization (Khatami *et al.*, 2017).

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was performed to determine the functional groups in the *O. sanctum* extract responsible for reducing and stabilizing the AgNPs. Spectra were recorded in the range of 400–4000 cm^{-1}. Key peaks corresponding to –OH (hydroxyl), –C=O (carbonyl), –NH (amine), and –C–O–C (ether) stretching vibrations were observed, indicating the involvement of phenolics, flavonoids, and proteins in nanoparticle formation. The shifts in peak intensities and positions between the extract and AgNPs confirmed capping and binding interactions (Khatami *et al.*, 2017).

X-ray Diffraction (XRD) Analysis

To determine the crystalline nature of the nanoparticles, XRD patterns were recorded using an X-ray diffractometer with Cu-Kα radiation (λ = 1.5406 Å). The characteristic Bragg reflections at 2θ values of approximately 38.1°, 44.3°, 64.5°, and 77.4° corresponded to the (111), (200), (220), and (311) planes of face-centered cubic (fcc) silver. The crystallite size was estimated using the Scherrer equation, which revealed an average size in the range of 15–25 nm, corroborating TEM findings (Khatami *et al.*, 2017).

Transmission Electron Microscopy (TEM)

TEM was conducted to directly observe the morphology and size distribution of the synthesized AgNPs. The images revealed predominantly spherical and uniformly dispersed nanoparticles, with particle sizes ranging from 12 to 30 nm depending on the formulation. The use of higher extract volumes led to the formation of smaller, more monodispersed particles, likely due to increased availability of capping agents from the plant extract. No evidence of significant aggregation was noted, indicating good stability of the biosynthesized nanoparticles (Khatami *et al.*, 2017).

2.5 Antibacterial Activity Assay

The antibacterial efficacy of the biosynthesized AgNPs was assessed using the agar well diffusion method. Clinical isolates of *Escherichia coli*, *Salmonella typhi*, and *Vibrio cholerae* were obtained from the microbiology laboratory (Sourced from National Institute of Cell Sciences, Pune). The bacterial cultures were grown overnight in nutrient broth and adjusted to a turbidity of 0.5 McFarland standard. Mueller-Hinton agar plates were inoculated with the bacterial suspension using sterile swabs. Wells (6 mm diameter) were punched into the agar, and 100 µL of AgNP suspension (100 µg/mL) was introduced into each well. Plates were incubated at 37°C for 24 hours, and zones of inhibition were measured in millimeters (Kalemba & Kunicka, 2003; Salina *et al.*, 2019; Yazdıç *et al.*, 2023).

2.6 Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical significance was evaluated using one-way ANOVA followed by Tukey's post-hoc test, with p-values < 0.05 considered significant. Data were processed using GraphPad Prism version 8.

3. RESULTS

3.1 Visual Observation and UV-Visible Spectroscopy

The synthesis of silver nanoparticles was initially indicated by a color change in the reaction mixture. All five formulations (F1–F5) exhibited a gradual shift from pale yellow to dark brown within 24 hours of incubation, suggesting the reduction of Ag^+ ions to Ag^0 nanoparticles by phytochemicals present in *Ocimum sanctum* extract. The intensity of the brown coloration increased with higher extract concentrations. UV-Vis spectroscopic analysis revealed characteristic surface plasmon resonance (SPR) peaks between 423 and 438 nm. The position of the SPR peak slightly red-shifted with increasing extract concentration, indicating the influence of capping agents on particle size and uniformity. F1 exhibited a peak at 423 nm, while F5 displayed a peak at 438 nm, supporting the formation of stable AgNPs across all formulations.

Table 2: Visual Observation and UV–Visible Spectroscopy of AgNP Formulations

Formulation Code	Extract Volume (mL)	Colour Change	SPR Peak (nm)
F1	5	Light brown	423
F2	10	Brown	426
F3	15	Dark brown	430
F4	20	Intense brown	435
F5	25	Very dark brown	438

Legend: SPR – Surface Plasmon Resonance.

3.2 FTIR Analysis

FTIR spectra of the *O. sanctum* extract and AgNPs demonstrated notable shifts in peak positions, confirming the involvement of plant biomolecules in nanoparticle synthesis and stabilization. Prominent peaks observed around 3325 cm^{-1} (–OH stretching), 1630 cm^{-1} (C=O stretching), and 1385 cm^{-1} (C–N bending) suggested the presence of alcohols, phenols, proteins, and flavonoids, which likely served as both reducing and capping agents.

Table 3: FTIR Analysis of *Ocimum sanctum* Extract and Synthesized AgNPs

Wavenumber (cm^{-1})	Functional Group	Assignment	Shift Observed in AgNPs
~3325	O–H stretch (phenols, alcohols)	Hydrogen-bonded –OH	Shifted to ~3300
~1630	C=O stretch (proteins, flavonoids)	Amide I (carbonyl vibration)	Shifted to ~1620
~1385	C–N bend (amines)	Aromatic amine or protein involvement	Slight shift to ~1372
~1050	C–O stretch (ethers, polysaccharides)	Alcohol/ether group	Shifted to ~1035

Legend: FTIR – Fourier Transform Infrared Spectroscopy; AgNPs – Silver Nanoparticles

3.3 XRD Analysis

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XRD patterns of the biosynthesized AgNPs showed distinct diffraction peaks at 2θ values corresponding to 38.1°, 44.3°, 64.5°, and 77.4°, matching the (111), (200), (220), and (311) planes of face-centered cubic (fcc) silver. These results confirmed the crystalline nature of the synthesized nanoparticles. The average crystallite size calculated using the Scherrer equation was approximately 18.6 nm.

Table 4: XRD Analysis of Biosynthesized Silver Nanoparticles

2θ (Degree)	Miller Indices (hkl)	Observed Peak Intensity	Phase Assignment
38.1°	(111)	Strong	Face-centered cubic (fcc) Ag
44.3°	(200)	Moderate	fcc Ag
64.5°	(220)	Weak	fcc Ag
77.4°	(311)	Weak	fcc Ag
Average Crystallite Size (by Scherrer Equation): 18.6 nm			

TEM Analysis

Transmission Electron Microscopy confirmed that the synthesized silver nanoparticles were spherical and moderately uniform in size distribution. The size of nanoparticles ranged from 12 to 30 nm across formulations. A slight decrease in average particle size was noted with increasing extract concentration, supporting the hypothesis that higher phytochemical content facilitated better capping and size control.

Table 5: TEM Analysis – Particle Size and Morphology of AgNP Formulations

Formulation Code	Observed Particle Shape	Particle Size Range (nm)	Average Particle Size (nm)
F1	Mostly spherical	25 – 32	28.4 ± 1.6
F2	Spherical to oval	22 – 30	25.2 ± 1.3
F3	Uniformly spherical	18 – 26	22.1 ± 1.2
F4	Spherical	15 – 22	18.7 ± 1.1
F5	Well-defined spherical	12 – 20	15.6 ± 1.4

Legend: TEM – Transmission Electron Microscopy; Values are mean ± SD based on n = 3 images.

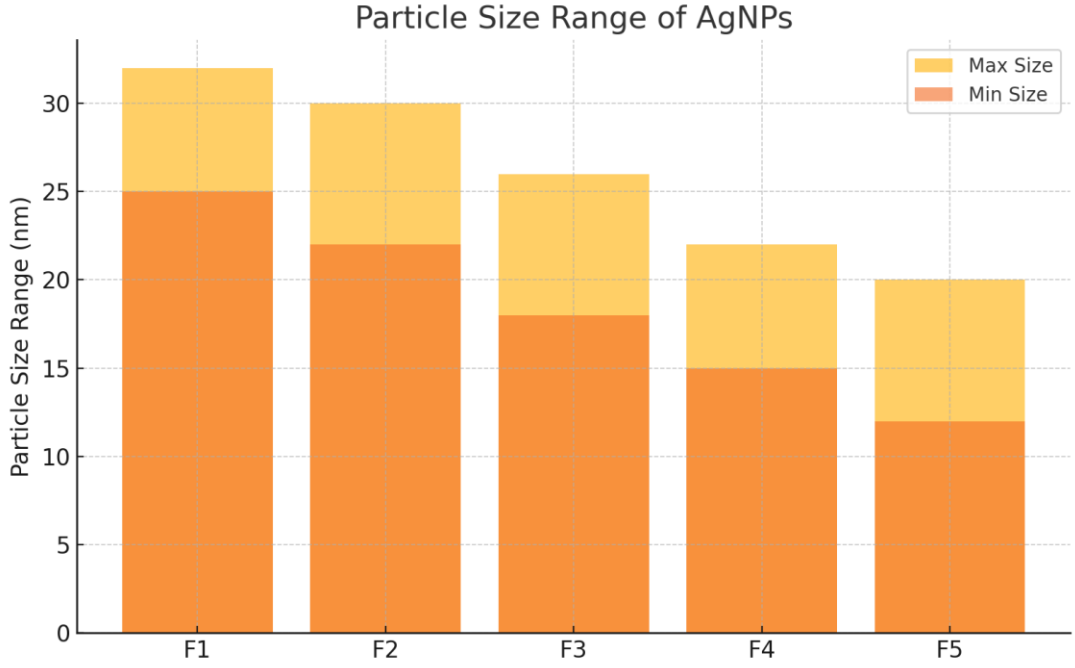

Figure 1. Particle Size (nm) range of AgNP Formulations

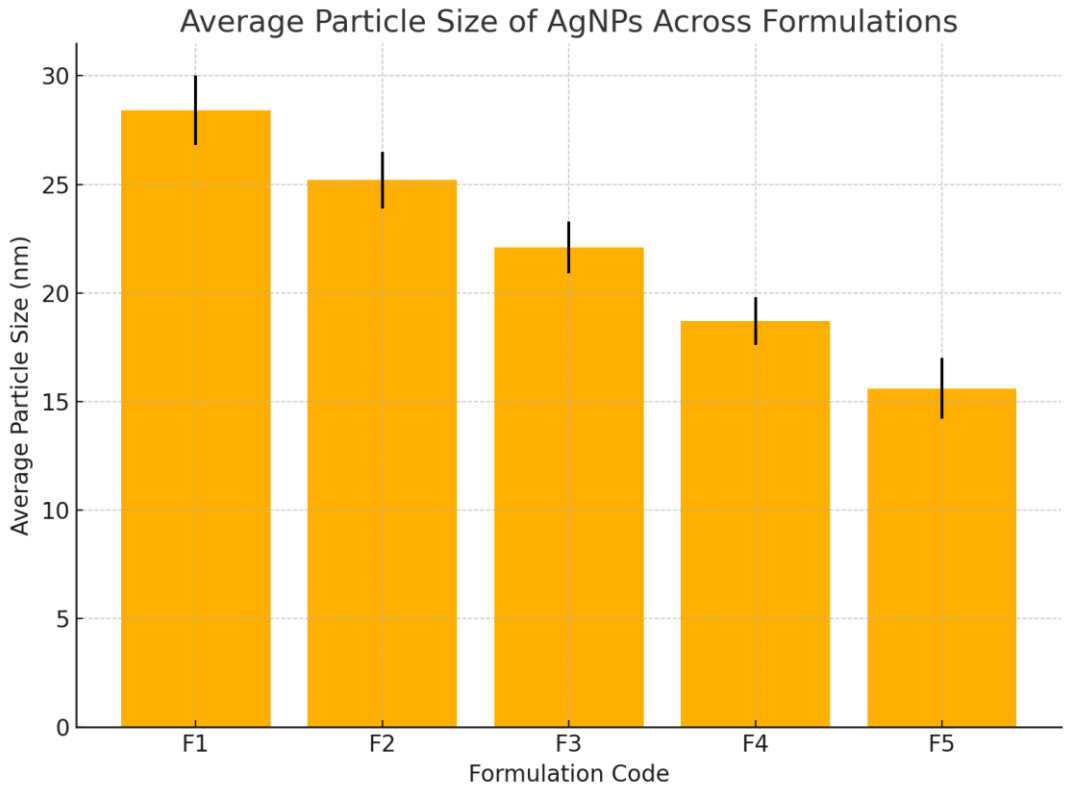

Figure 2. Average Particle Size (nm) of AgNP Formulations

3.5 Antibacterial Activity

All AgNP formulations exhibited significant antibacterial activity against *Escherichia coli*, *Salmonella typhi*, and *Vibrio cholerae* as measured by the agar well diffusion method. Zones of inhibition ranged from 14.2 mm (F1 against *S. typhi*) to 22.1 mm (F5 against *E. coli*). F5 demonstrated the highest antibacterial efficacy, which may be attributed to smaller particle size and greater surface reactivity.

Table 6: Antibacterial Activity of AgNP Formulations Against Waterborne Pathogens (Zone of Inhibition in mm)

Formulation Code	*E. coli* (mm)	*S. typhi* (mm)	*V. cholerae* (mm)
F1	14.8 ± 0.5	14.2 ± 0.4	15.1 ± 0.6
F2	16.5 ± 0.4	15.6 ± 0.5	16.7 ± 0.3
F3	18.9 ± 0.6	17.4 ± 0.3	18.5 ± 0.4
F4	20.6 ± 0.5	19.2 ± 0.4	20.3 ± 0.5
F5	22.1 ± 0.3	21.0 ± 0.5	21.7 ± 0.4
Control ($AgNO_3$ only)	9.3 ± 0.4	8.7 ± 0.6	9.5 ± 0.3

Legend: Values represent mean ± SD (n = 3); Agar well diffusion method.

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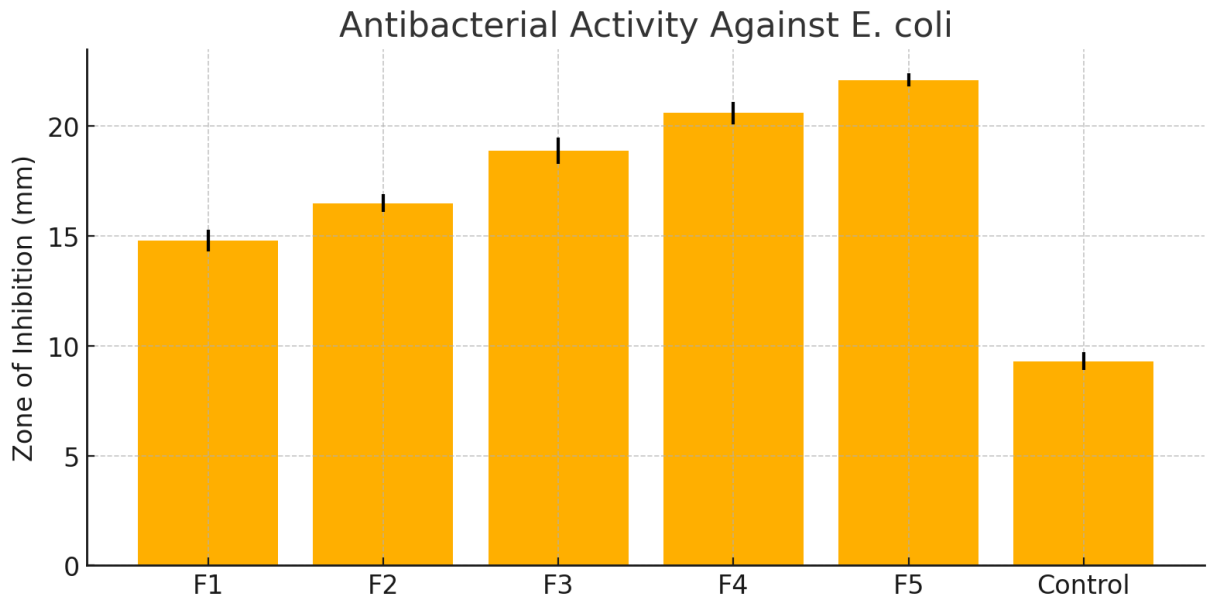

Figure 3. Antibacterial Activity of AgNP Formulations Against Waterborne Pathogens, *E. coli* (Zone of Inhibition in mm)

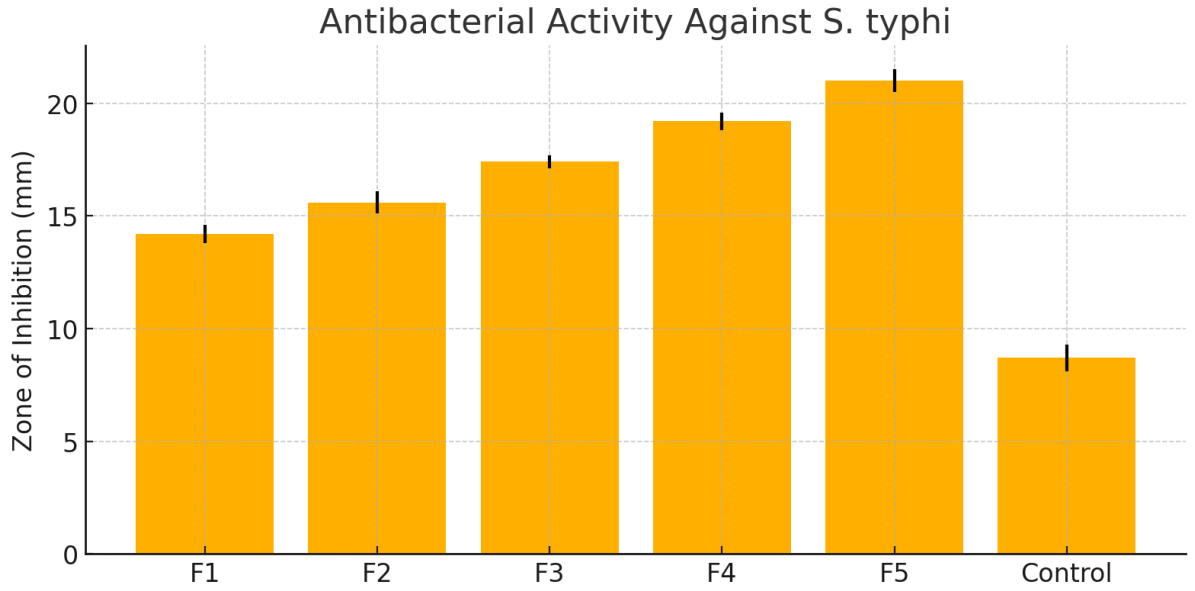

Figure 4. Antibacterial Activity of AgNP Formulations Against Waterborne Pathogens, *S. typhi* (Zone of Inhibition in mm)

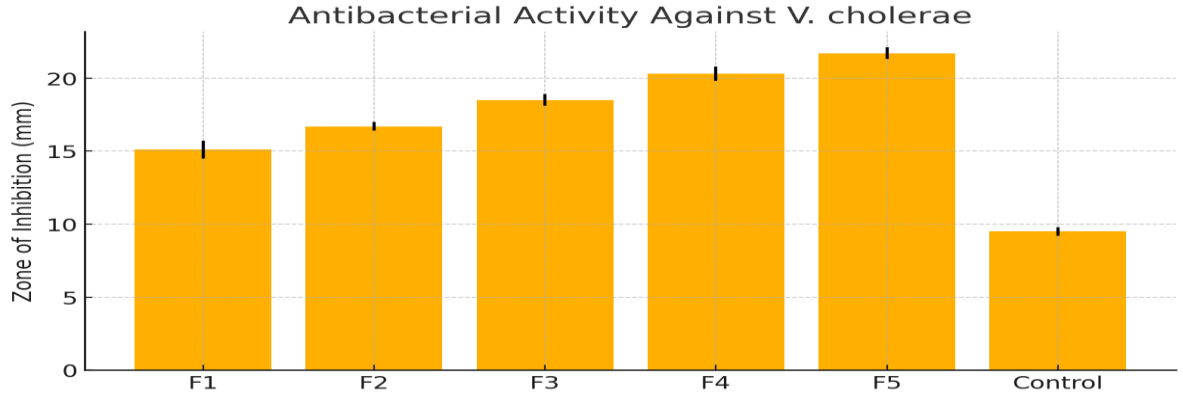

Figure 5. Antibacterial Activity of AgNP Formulations Against Waterborne Pathogens, *V.cholerae* (Zone of Inhibition in mm)

4. DISCUSSION

The present study successfully demonstrated the eco-friendly synthesis of silver nanoparticles (AgNPs) using *Ocimum sanctum* leaf extract, highlighting the potential of green nanotechnology in addressing microbial contamination in water. The color transformation from pale yellow to varying intensities of brown across formulations was a preliminary visual indicator of nanoparticle formation, attributed to the surface plasmon resonance (SPR) phenomenon typical of silver nanoparticles. The corresponding UV-Vis spectral peaks between 423 and 438 nm further confirmed nanoparticle synthesis, with the slight red shift in higher extract concentrations indicating smaller and more stabilized nanoparticles due to increased availability of phytochemicals acting as reducing and capping agents.

FTIR spectral analysis provided insight into the biomolecules involved in nanoparticle synthesis. Functional groups such as hydroxyl, carbonyl, amine, and ether were evident, suggesting the presence of flavonoids, phenolics, and proteins that facilitated both reduction of Ag^+ ions and stabilization of the resulting nanoparticles. The reduction process is consistent with previous findings that plant-based polyphenols and flavonoids can act as electron donors, converting silver ions into elemental silver (Ag^0) while simultaneously stabilizing the nanoparticles against aggregation. XRD analysis confirmed the crystalline nature of the synthesized AgNPs, with distinct Bragg peaks corresponding to the face-centered cubic (fcc) structure of silver. The crystallite size calculated using the Scherrer equation averaged around 18.6 nm, supporting the data observed under TEM. Transmission Electron Microscopy revealed well-dispersed, spherical nanoparticles, with particle size decreasing as the volume of plant extract increased. This inverse relationship between extract concentration and particle size aligns with the theory that higher concentrations of stabilizing agents lead to better control over nucleation and growth phases during synthesis. Antibacterial assays showed that the biosynthesized AgNPs exhibited potent inhibitory effects against *Escherichia coli*, *Salmonella typhi*, and *Vibrio cholerae*. The antibacterial efficacy improved progressively from F1 to F5, correlating with reduced particle size and higher phytochemical content. The F5 formulation, which contained the highest volume of *O. sanctum* extract, displayed the largest zones of inhibition across all tested strains. This enhanced activity is likely due to the increased surface area of smaller nanoparticles, which improves their ability to interact with and disrupt bacterial membranes. Additionally, the presence of residual plant phytochemicals on the nanoparticle surface may contribute synergistically to antibacterial action.

These findings are in agreement with several previous studies that reported the effectiveness of green-synthesized silver nanoparticles against a wide range of bacterial pathogens. Notably, the use of *O. sanctum* introduces an added advantage due to its known antimicrobial properties, which may enhance the therapeutic potential of the formulation. Overall, the study reinforces the dual functionality of *O. sanctum* in nanoparticle synthesis and antimicrobial action. The simplicity, cost-effectiveness, and scalability of this green method make it a promising alternative for developing antimicrobial agents for water disinfection, particularly in resource-limited settings. However, further in vivo and toxicity studies are necessary to validate the safety and long-term stability of these biosynthesized nanoparticles before practical applications in water purification systems.

5. CONCLUSIONS

This study successfully demonstrated the green synthesis of silver nanoparticles using *Ocimum sanctum* leaf extract, presenting a sustainable, low-cost, and non-toxic alternative to conventional nanoparticle fabrication methods. The visual and spectroscopic confirmation of nanoparticle formation, along with FTIR, XRD, and TEM characterization, validated the involvement of plant-derived biomolecules in reducing and capping silver ions, leading to the generation of stable, spherical, and crystalline AgNPs. The size and dispersion of the particles were favorably influenced by the volume of extract used, with higher concentrations producing smaller and more uniform nanoparticles. The synthesized AgNPs exhibited strong antibacterial activity against major waterborne pathogens, including *E. coli*, *S. typhi*, and *V. cholerae*. The enhanced efficacy of the F5 formulation highlighted the role of phytochemical content in optimizing nanoparticle functionality. These findings reinforce the potential of integrating phytomedicine and nanotechnology to address environmental health challenges, particularly in regions where waterborne diseases are prevalent, and resources for chemical disinfection are limited. Future work

should focus on assessing the long-term stability, cytotoxicity, and environmental safety of these nanoparticles in real-world water treatment systems. Nonetheless, this study lays a strong foundation for the application of *O. sanctum*-based AgNPs in eco-friendly water purification technologies.

REFERENCES

1. Cohen, M. M. (2014). Tulsi - Ocimum sanctum: A herb for all reasons. *J Ayurveda Integr Med*, *5*(4), 251-259. https://doi.org/10.4103/0975-9476.146554
2. Gahlawat, G., Shikha, S., Chaddha, B. S., Chaudhuri, S. R., Mayilraj, S., & Choudhury, A. R. (2016). Microbial glycolipoprotein-capped silver nanoparticles as emerging antibacterial agents against cholera. *Microb Cell Fact*, *15*, 25. https://doi.org/10.1186/s12934-016-0422-x
3. Islam, S. N., Naqvi, S. M. A., Parveen, S., & Ahmad, A. (2021). Application of mycogenic silver/silver oxide nanoparticles in electrochemical glucose sensing; alongside their catalytic and antimicrobial activity. *3 Biotech*, *11*(7), 342. https://doi.org/10.1007/s13205-021-02888-4
4. Jiang, J., Zhang, X., Wen, G., Zhu, M., & Zheng, Y. (2024). Purification Resistance Index: A new water quality assessment method toward drinking water production. *Water Res*, *267*, 122555. https://doi.org/10.1016/j.watres.2024.122555
5. Kalemba, D., & Kunicka, A. (2003). Antibacterial and antifungal properties of essential oils. *Curr Med Chem*, *10*(10), 813-829. https://doi.org/10.2174/0929867033457719
6. Khatami, M., Mortazavi, S. M., Kishani-Farahani, Z., Amini, A., Amini, E., & Heli, H. (2017). Biosynthesis of Silver Nanoparticles Using Pine Pollen and Evaluation of the Antifungal Efficiency. *Iran J Biotechnol*, *15*(2), 95-101. https://doi.org/10.15171/ijb.1436
7. Khatoon, S., Kalam, N., Balasubramaniam, V. R., Shaikh, M. F., & Ansari, M. T. (2022). Chemotherapeutic Role of Polyphenols Present in Ocimum sanctum. *Anticancer Agents Med Chem*, *22*(20), 3325-3342. https://doi.org/10.2174/1871520622666220516142839
8. Kumar, P., & Patel, D. (2023). Ocimum Sanctum: An All-Round Treatment for Cancer? *Altern Ther Health Med*, *29*(4), 253-257.
9. Kumari, S., Singh, P. A., Hazra, S., Sindhwani, R., & Singh, S. (2024). Ocimum sanctum: The Journey from Sacred Herb to Functional Food. *Recent Adv Food Nutr Agric*, *15*(2), 83-102. https://doi.org/10.2174/012772574x290140240130101117
10. Kumari, T., Phogat, D., & Shukla, V. (2023). Exploring the multipotentiality of plant extracts for the green synthesis of iron nanoparticles: A study of adsorption capacity and dye degradation efficiency. *Environ Res*, *229*, 116025. https://doi.org/10.1016/j.envres.2023.116025
11. Küp, F., Çoşkunçay, S., & Duman, F. (2020). Biosynthesis of silver nanoparticles using leaf extract of Aesculus hippocastanum (horse chestnut): Evaluation of their antibacterial, antioxidant and drug release system activities. *Mater Sci Eng C Mater Biol Appl*, *107*, 110207. https://doi.org/10.1016/j.msec.2019.110207
12. Lam, I. T. Y., Choi, S. J., Lu, D., & Kim, Y. (2023). Functionalized metal-organic frameworks for heavy metal ion removal from water. *Nanoscale*, *15*(24), 10189-10205. https://doi.org/10.1039/d3nr02250a
13. Madikizela, L. M., & Pakade, V. E. (2023). Trends in removal of pharmaceuticals in contaminated water using waste coffee and tea-based materials with their derivatives. *Water Environ Res*, *95*(4), e10857. https://doi.org/10.1002/wer.10857
14. Nan, Y., Gomez-Maldonado, D., Whitehead, D. C., Yang, M., & Peresin, M. S. (2023). Comparison between nanocellulose-polyethylenimine composites synthesis methods towards multiple water pollutants removal: A review. *Int J Biol Macromol*, *232*, 123342. https://doi.org/10.1016/j.ijbiomac.2023.123342
15. R, C., R, S., Mohideen, K., Nandagopal, P., Jayamani, L., & Jeyakumaran, S. (2022). Anti-candidal Effect of Ocimum sanctum: A Systematic Review on Microbial Studies. *Cureus*, *14*(5), e24749. https://doi.org/10.7759/cureus.24749
16. Salina, E. G., Ekins, S., & Makarov, V. A. (2019). A rapid method for estimation of the efficacy of potential antimicrobials in humans and animals by agar diffusion assay. *Chem Biol Drug Des*, *93*(6), 1021-1025. https://doi.org/10.1111/cbdd.13427
17. Saravanan, A., Ragini, Y. P., Kumar, P. S., Thamarai, P., & Rangasamy, G. (2023). A critical review on the removal of toxic pollutants from contaminated water using magnetic hybrids. *Environ Sci Pollut Res Int*, *30*(48), 105099-105118. https://doi.org/10.1007/s11356-023-29811-z
18. Swamy, M. K., Akhtar, M. S., Mohanty, S. K., & Sinniah, U. R. (2015). Synthesis and characterization of silver nanoparticles using fruit extract of Momordica cymbalaria and assessment of their in vitro antimicrobial, antioxidant and cytotoxicity activities. *Spectrochim Acta A Mol Biomol Spectrosc*, *151*, 939-944. https://doi.org/10.1016/j.saa.2015.07.009
19. Tamboli, D. P., & Lee, D. S. (2013). Mechanistic antimicrobial approach of extracellularly synthesized silver nanoparticles against gram positive and gram negative bacteria. *J Hazard Mater*, *260*, 878-884. https://doi.org/10.1016/j.jhazmat.2013.06.003
20. Xia, C., Li, X., Wu, Y., Suharti, S., Unpaprom, Y., & Pugazhendhi, A. (2023). A review on pollutants remediation competence of nanocomposites on contaminated water. *Environ Res*, *222*, 115318. https://doi.org/10.1016/j.envres.2023.115318
21. Yazdıç, F. C., Karaman, A., Torğut, G., & Ayhan, N. K. (2023). Antibacterial activity of novel synthesized chitosan-graft-poly(N-tertiary butylacrylamide)/neodymium composites for biomedical application. *J Basic Microbiol*, *63*(9), 1049-1056. https://doi.org/10.1002/jobm.202300004