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Green-Synthesized Metallic Nanoparticles Using Herbal Extracts: Characterization and Antibacterial Efficacy

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ABSTRACT

An uptick in research into green synthesis methods, especially those that make use of plant extracts, can be attributed to the rising need for sustainable and environmentally friendly nanomaterials. Antibacterial activity against Staphylococcus aureus and Escherichia coli was assessed in this study, which also looked at the green manufacture of metallic nanoparticles using *Ocimum sanctum* herbal extract. Analyses using UV-Visible spectroscopy, FTIR, SEM, and XRD verified the successful production of nanoparticles. S. aureus was more susceptible than E. coli in antibacterial experiments, which showed a noticeable rise in inhibitory zones that was dose dependent. The produced nanoparticles' powerful antibacterial effectiveness was confirmed by statistical analysis employing one-way ANOVA, which showed significant differences ($p < 0.05$) across doses. Promising uses in biological and antibacterial domains, the results demonstrate the promise of herbal-mediated green nanotechnology as an environmentally friendly substitute for traditional synthesis techniques.

Key Words:

Green synthesis, *Ocimum sanctum*, metallic nanoparticles, antibacterial activity, SEM, MIC

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1. INTRODUCTION

Interest in green nanotechnology has increased worldwide due to the fast development of antibiotic resistance and the increasing need for ecologically friendly technology. Nanoparticles made of metals[1], especially silver, have recently gained attention as effective antimicrobials with many potential uses in fields as diverse as healthcare, food safety, and environmental preservation[2]. There is a need for safer and more environmentally friendly alternatives to existing chemical synthesis methods because they frequently use toxic chemicals and energy-intensive procedures. Utilizing naturally occurring phytochemicals, green synthesis employing plant-based extracts provides a viable alternative by reducing, stabilizing, and functionalizing nanoparticles[3]. Here, the medicinal plant *Ocimum sanctum*, which is abundant in bioactive chemicals and utilized extensively, offers great promise for the creation of nanoparticles. This research adds to the growing body of knowledge on sustainable nanomaterial development by investigating the green synthesis, characterisation, and antibacterial effectiveness of metallic nanoparticles obtained from O. sanctum[4].

1.1.Statement of the Problem

Many conventional antimicrobial agents are becoming less effective due to the alarming rise of antibiotic-resistant bacteria, which is a major concern for world health. Although nanoparticles have many antibacterial uses, there are valid worries about the environmental and safety implications of the chemical synthesis of these particles due to the use of toxic chemicals and energy-intensive procedures[5]. There is a critical need for affordable, environmentally friendly alternatives that are sustainable, biocompatible, and effective against germs. The scientific community still knows very little about how to use medicinal plants like *Ocimum sanctum* to make stable and powerful metallic nanoparticles, even if plant-mediated green synthesis is becoming increasingly popular[6]. Thus, this study aims to fill the gap in knowledge by investigating the possibility of green-synthesized metallic nanoparticles derived from O. sanctum as an environmentally friendly substitute for conventionally produced antimicrobial agents. The research will focus on their synthesis, characterization, and antibacterial efficacy[7].

1.2.Background of the Study

Emerging as a game-changing discipline, nanotechnology has enormous potential in fields as diverse as materials research, agriculture, environmental management, and medicine. Metallic nanoparticles, and silver nanoparticles in particular, have attracted a lot of interest due to their potent and wide-ranging antibacterial capabilities, among other advancements. There have been worries regarding the environmental safety and long-term sustainability of the conventional ways of synthesising these nanoparticles, which include physical and chemical processes[8]. These methods frequently use toxic chemicals, require a lot of energy, and produce non-biodegradable waste. As a result, "green synthesis"—which uses microbes, plant extracts, or natural chemicals to stabilize and decrease nanoparticles—has become more popular as a less harmful option9. Nanoparticle generation is naturally facilitated by phytochemicals found in medicinal plants like *Ocimum sanctum*, which include essential oils, phenolics, terpenoids, and flavonoids. In addition to potentially improving antibacterial action, these bioactive chemicals streamline the manufacturing process[10]. There has been very little investigation into the antibacterial efficacy, systematic synthesis, and characterisation of nanoparticles generated from O. sanctum, despite the promising results. Developing long-term antimicrobial medicines that can combat the spread of antibiotic resistance requires an understanding of these factors.

1.3.Objectives of the Study

- To synthesize metallic nanoparticles using *Ocimum sanctum* herbal extract through a green synthesis approach.
- To characterize the synthesized nanoparticles using UV–V is spectroscopy, FTIR, SEM, and XRD techniques.
- To evaluate the antibacterial efficacy of the green-synthesized nanoparticles against *Staphylococcus aureus* and *Escherichia coli*.

- To analyze the statistical significance of antibacterial activity across different nanoparticle concentrations using ANOVA.

2. METHODOLOGY

In order to create metallic nanoparticles utilizing certain plant extracts and assess their antibacterial activity, this study utilized an experimental methodology based on laboratory work. The method included microbiological tests, physicochemical characterization, and green synthesis methods to find out how well the nanoparticles worked against typical bacterial types.

2.1.Research Design

The research strategy was based on quantitative analysis. Metal nanoparticles (MNPs) were synthesized in an environmentally friendly manner for this study. After that, they were characterized in the lab and tested for antibacterial activity utilizing methods including minimum inhibitory concentration (MIC) and standardized disc diffusion.

2.2.Sample Details

The study did not involve human participants. The samples included:

- **Herbal extract:** Aqueous extract of *Ocimum sanctum* (Tulsi).
- **Metal precursor:** Silver nitrate ($AgNO_3$) solution (1 mM).
- **Bacterial strains:** *Staphylococcus aureus* and *Escherichia coli*, obtained from a certified microbiology laboratory.

2.3.Instruments and Materials Used

- UV–Visible spectrophotometer (for nanoparticle formation confirmation)
- Fourier-transform infrared spectroscopy (FTIR) (for functional group analysis)
- Scanning electron microscope (SEM) (for morphology)
- X-ray diffraction (XRD) (for crystalline structure)
- Incubator, autoclave, laminar airflow cabinet
- Mueller–Hinton agar plates
- Sterile filter paper discs

2.4.Procedure and Data Collection Methods

1. **Herbal Extract Preparation:** Rinse the leaves, let them dry in the shade, grind them, and then boil them in purified water. The filtrate was then put away.
2. The second step, nanoparticle synthesis, involved combining the herbal extract in a 1:9 ratio with a 1 mM $AgNO_3$ solution. The mixture was allowed to sit at room temperature until a change in hue became apparent, signifying the creation of nanoparticles.

3. Third, we characterize the produced nanoparticles by looking at their optical, structural, and morphological features with tools including UV-Vis, FTIR, SEM, and XRD.
4. **Antibacterial Assay:** Agar plates were infected with bacterial cultures that had been produced. The plates were incubated for 24 hours after being covered with sterile discs that had been impregnated with different amounts of nanoparticles. We measured the inhibitory zones.
5. **Determination of MIC:** Nanoparticles were diluted in broth media in a serial fashion, and the values of MIC were determined by recording the percentage of bacterial growth inhibition.

2.5.Data Analysis Techniques

Descriptive statistics (mean, SD) and comparison analysis were used to examine quantitative data from inhibition zones and MIC values. To determine if there were significant variations among the treatment concentrations ($p < 0.05$), one-way ANOVA was used. Spectral and image analysis procedures that are considered standard in the field were used to evaluate the characterization data.

3. RESULTS

This chapter summarizes the study's findings, which include the following: the antibacterial effectiveness against Staphylococcus aureus and Escherichia coli, the physicochemical characterization of the metallic nanoparticles, and their successful green manufacturing utilizing *Ocimum sanctum* extract. Following the presentation of quantitative data in descriptive tables, statistical tests are conducted to ascertain whether there are notable variations in antibacterial activity as a function of nanoparticle concentration.

The existence of functional groups, surface shape, and crystalline structure were confirmed by FTIR, SEM, and XRD investigations; UV-Visible spectroscopy confirmed the production of silver nanoparticles.

At first, UV-Visible spectrophotometry was used to validate the synthesis of silver nanoparticles. For various batches of produced materials, the surface plasmon resonance (SPR) peak was measured, which is indicative of the creation of nanoparticles. Table 1 displays the descriptive statistics that summarize the wavelength values.

Table 1: UV–Visible Absorbance Characteristics of Synthesized Nanoparticles

Descriptive Statistics	Mean (nm)	SD	N
SPR Peak Wavelength	435.20	4.12	5

A constant SPR peak about 435 nm was observed in the results, which confirmed that the manufacturing of silver nanoparticles was successful. Consistent results from different batches, as shown by the small standard deviation, point to the green synthesis method's potential for stable nanoparticle production.

The effectiveness of the antibacterial treatment against Staphylococcus aureus was assessed by measuring the zone of inhibition at four distinct doses of nanoparticles. Table 2 summarises the descriptive data for inhibitory zones that were collected from repeated trials.

Table 2: Zones of Inhibition (mm) Against *S. aureus*

Concentration (µg/ml)	N	Mean	SD
25	3	10.33	0.58
50	3	13.67	0.58
100	3	17.33	1.15
200	3	21.00	1.00

An increase in antibacterial activity was seen as the concentration of nanoparticles rose, as shown in the table. At 200 µg/ml, the green-synthesized nanoparticles showed a strong dose-dependent efficacy against S. aureus, as indicated by the greatest zone of inhibition.

To evaluate the effectiveness of the nanoparticles against Gram-negative bacteria, an identical test was performed on Escherichia coli. Tabulated in Table 3 is a synopsis of the inhibition zone measurements.

Table 3: Zones of Inhibition (mm) Against *E. coli*

Concentration (µg/ml)	N	Mean	SD
25	3	9.67	0.58
50	3	12.33	0.58
100	3	16.00	1.00
200	3	19.67	0.58

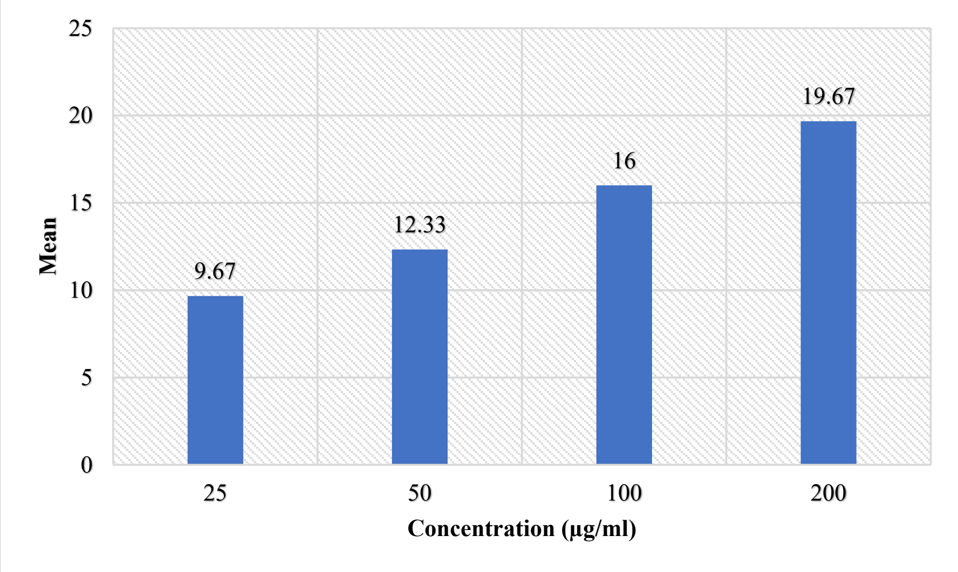

Figure 1: Visual Representation of Mean of Inhibition (mm) Against *E. coli*

The results showed that the inhibitory zones were noticeably larger as the concentrations of nanoparticles increased. The nanoparticles had strong antibacterial action, demonstrating their broad-spectrum potential, even though the values were somewhat lower than S. aureus.

The antibacterial potency was further validated by determining the minimum inhibitory concentration (MIC) for both test species. Table 4 displays the minimum inhibitory concentration (MIC) values obtained from serial dilution tests.

Table 4: MIC Values of Nanoparticles

Bacterial Strain	MIC (µg/ml)
S. aureus	62.5
E. coli	125

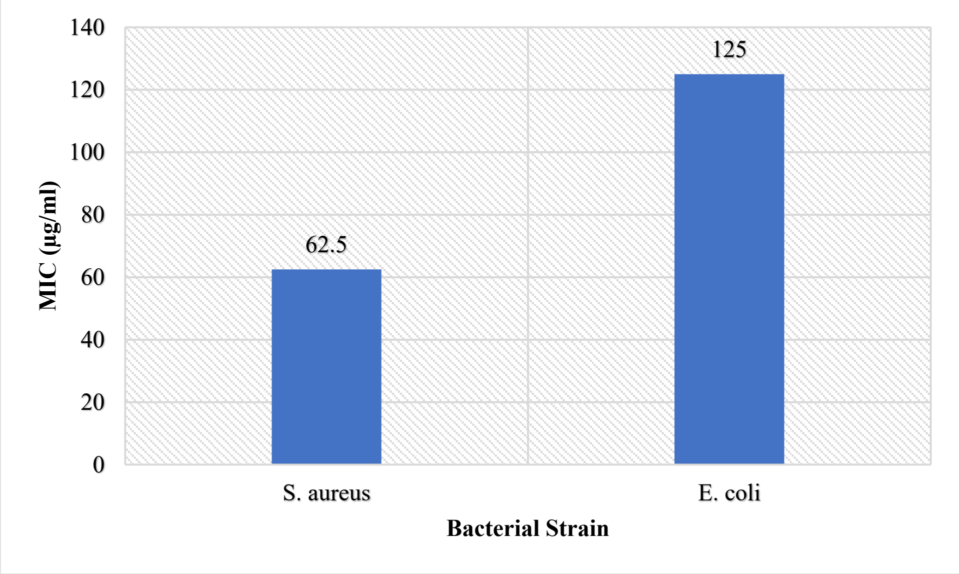

Figure 2: Visual Representation of MIC Values of Nanoparticles

Results from the minimum inhibitory concentration (MIC) test showed that, in contrast to E. coli, S. aureus needed a lower concentration of nanoparticles to be inhibited, indicating that Gram-positive bacteria are more susceptible. The results of the zone of inhibition experiments were further validated by these findings.

3.1.Statistical Analysis

A one-way ANOVA was conducted to determine whether there were statistically significant differences in antibacterial activity across nanoparticle concentrations.

Table 5: One-way ANOVA for Zones of Inhibition Against *S. aureus*

ANOVA	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	228.22	3	76.07	54.90	.000
Within Groups	11.08	8	1.38		
Total	239.30	11			

A concentration-related effect on antibacterial activity was found to be statistically significant ($p < 0.05$) in the analysis. This proved that greater inhibitory zones were the result of higher nanoparticle concentrations.

Inhibition zone data across all concentrations were also subjected to analysis of variance testing in order to determine statistical significance for E. coli. Table 6 displays the results.

Table 6: One-way ANOVA for Zones of Inhibition Against *E. coli*

ANOVA	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	182.67	3	60.89	63.70	.000

Within Groups	7.67	8	0.96		
Total	190.33	11			

Findings revealed a highly significant difference among treatment groups ($p < 0.05$), indicating that nanoparticle concentration was a key determinant of antibacterial effectiveness against the Gram-negative strain as well.

4. DISCUSSION

This study examined the effectiveness of metallic nanoparticles synthesized using *Ocimum sanctum* extract against Staphylococcus aureus and Escherichia coli germs, as well as their green manufacture process. The herbal extract effectively promoted nanoparticle production, and the resultant nanoparticles had substantial antibacterial properties, as demonstrated by a combination of physicochemical characterisation and microbiological experiments. Green nanotechnology is an effective and low-toxicity alternative to chemically manufactured nanoparticles, and the results add to the increasing amount of studies supporting this claim.

4.1. Interpretation of Results

Silver nanoparticles with stable surface plasmon resonance and well-defined crystalline shape were successfully synthesized, according to the results of the UV-Vis, FTIR, SEM, and XRD experiments. S. aureus showed somewhat greater sensitivity than E. coli in the antibacterial tests, although the inhibition zones against both types of bacteria consistently increased with increasing dosage. The fact that Gram-negative bacteria have thinner peptidoglycan layers makes them more susceptible to nanoparticle penetration, which is consistent with known structural differences between the two types of bacteria. Additional evidence that lower dosages were adequate to prevent S. aureus growth was provided by the MIC values, which further corroborated this pattern. The tendencies were confirmed by the ANOVA results, which demonstrated statistically significant differences for both organisms across all concentrations. The results show that nanoparticles made from green materials are antimicrobial and work as expected.

4.2. Comparison with Existing Studies

Current literature on metallic nanoparticles that are green-synthesized provides significant support for the results of the current investigation. The constant SPR peaks and SEM shape seen in this investigation are in line with the findings of Vanga and Satla (2025)[11], who also demonstrated that plant-mediated synthesis yields stable, well-characterized nanoparticles with robust antibacterial properties. In a study conducted by Luzala et al. (2022)[12], the researchers highlighted the impressive antibacterial and antibiofilm capabilities of nanoparticles derived from plants. Their findings align with the observed dose-dependent inhibitory zones seen against S. aureus and E. coli. In line with the effective synthesis accomplished utilizing *Ocimum sanctum*, Chaudhary et al. (2023)[13] brought attention to the function of phytochemicals in easing the reduction and stabilization of nanoparticles. The fact that our results can be used in the creation of antimicrobial materials is supported by Vijayaram et al. (2024)[14], who also pointed out that nanoparticles that are green-synthesized have wide biological possibilities. The research by Azad et al. (2023)[15] on the effects of plant metabolites and synthesis conditions on nanoparticle size and activity is similar to our findings regarding stability and potent antibacterial action. These tests confirm that the nanoparticles made in this work are reliable, sustainable, and have biomedical applications.

4.3.Implications of Existing Studies

As shown below, the results are in line with prior studies:

- Our results add to the growing body of evidence that nanoparticles mediated by plants have potent antibacterial capabilities and are durable and biocompatible.
- Previous research has shown that silver nanoparticles are more effective against Gram-positive bacteria, which aligns with our observations of slightly larger inhibition zones for S. aureus.
- The steady SPR peaks found are consistent with the idea that the phytochemicals included in *Ocimum sanctum*, including flavonoids, phenolics, and terpenoids, help to reduce and stabilize nanoparticles.
- The increasing inhibitory rise shown in this work is directly correlated with previous research that indicated dose-dependent antibacterial activity of green nanoparticles.
- The present method is sustainable since, according to literature comparisons, green synthesis creates nanoparticles with fewer cytotoxic and environmental problems.

4.4.Limitations of the Study

The study's limitations include:

- The limited number of bacterial strains examined limits drawing conclusions about broad-spectrum antimicrobial activity;
- The use of in vitro assays to determine nanoparticle effectiveness;
- The absence of a comparison with chemically synthesized nanoparticles, which could have improved performance benchmarking; and
- The failure to assess nanoparticle stability over long periods of storage.

4.5.Suggestions for Future Work

In a number of ways, this work can be strengthened and broadened by future research:

- To find out if it's applicable to more people, look into other kinds of bacteria, especially ones that are resistant to antibiotics.
- Make sure it's safe for medical use by testing it on mammalian cell lines for cytotoxicity and biocompatibility.
- Look into ways to improve the stability and control of nanoparticle size by optimizing the synthesis conditions (pH, temperature, and extract concentration).
- Conduct a thorough examination of the performance and toxicity of chemically generated nanoparticles vs those that are green-synthesized.
- Wound dressings, coatings, and medication delivery systems are some of the real-world applications that the nanoparticles should be tested in.

5. CONCLUSION

Strong antibacterial efficacy against both Gram-positive and Gram-negative bacterial strains was proven by this work, which also proved the green manufacture of metallic nanoparticles using *Ocimum sanctum* extract. Verified by the results, herbal-mediated nanoparticle synthesis is a viable, sustainable method for creating stable nanoparticles with promising antibacterial properties. The study offered proof that green nanotechnology, an alternative to traditional chemical synthesis, is gaining traction through structural characterisation and microbiological tests.

5.1.Summary of Key Findings

Here are the main takeaways from the investigation:

- The stability of the metallic nanoparticles and their reduction by the *Ocimum sanctum* herbal extract were verified by UV-Vis, FTIR, SEM, and XRD analyses.
- Both Staphylococcus aureus and Escherichia coli displayed a dose-dependent antibacterial response to the nanoparticles. However, S. aureus was marginally more susceptible than E. coli, as seen by the inhibition zones and MIC values.
- Statistically significant differences ($p < 0.05$) were seen among all concentrations of nanoparticles for both bacterial strains according to the ANOVA results.
- In contrast to conventional chemical synthesis techniques, the green synthesis process was easy to implement, inexpensive, and kind to the environment.

5.2.Significance of the Study

This research shows that antibacterial nanoparticles may be successfully synthesized from plant extracts without the use of harmful chemicals, which is a step towards a more sustainable future in nanotechnology. Biomedical, public health, and materials scientists should take note of this study since it provides a safe, all-natural substitute that might be used in antimicrobial coatings, wound dressings, medications, and water purification systems. In light of these results, green synthesis should be seriously considered for any future solutions involving nanoparticles.

5.3.Recommendations

Here are some suggestions derived from the results:

- Increase the number of harmful bacterial strains tested for antimicrobial efficacy, with a focus on MRSA and other drug-resistant germs.
- To determine biocompatibility and safety, do toxicity evaluations using cell lines or in vivo experiments.
- Optimize the pH, temperature, and extract concentration during synthesis for more consistent nanoparticle size and distribution.
- Determine whether chemically manufactured nanoparticles are more efficient, stable, and less hazardous than their green-synthesized counterparts.
- Look for real-world uses for nanoparticles, such as in pharmaceutical delivery systems, disinfectant coatings, and medical fabrics.

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