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Process Optimization for Green Synthesis of Silver Nanoparticles using *Acalypha godseffiana* Leaves Extract and its Biological Activity Against MRSA

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) poses a serious global health threat due to its multidrug resistance, complicating treatments in both human and veterinary medicine. The rising challenge of antimicrobial resistance necessitates alternative therapeutic strategies, including nanotechnology-based interventions. In this study, silver nanoparticles (AgNPs) were biosynthesized, optimized, and characterized using *Acalypha godseffiana* leaf extract, a medicinal plant native to Nigeria, serving as a natural reducing and stabilizing agent. Optimal synthesis was achieved using 1 mM $AgNO_3$ at 90 °C for 90 minutes with 1 mg/mL of plant extract. UV–Vis spectroscopy confirmed AgNP formation with a surface plasmon resonance peak at 432 nm. Scanning electron microscopy showed spherical, well-dispersed particles with an average diameter of 37 nm. In comparison, X-ray diffraction revealed a face-centered cubic crystalline structure with a mean crystallite size of 29 nm. Energy-dispersive X-ray spectroscopy detected elemental silver with a prominent signal at 3.0–3.2 keV. A zeta potential of −57.5 mV indicated excellent colloidal stability. FTIR analysis identified functional groups such as O–H and C=O, suggesting phytochemical involvement in particle stabilization. The biosynthesized AgNPs exhibited significant antibacterial activity against MRSA, yielding a maximum inhibition zone of 20.78 mm, a minimum inhibitory concentration (MIC) of 625 µg/mL, and a minimum bactericidal concentration (MBC) of 1250 µg/mL. This study is the first to report the anti-MRSA activity of *A. godseffiana*-derived AgNPs, underscoring their potential as eco-friendly and effective antimicrobial agents against resistant pathogens.

Keywords: Green synthesis, Silver nanoparticles, *Acalypha godseffiana*, Methicillin-resistant *Staphylococcus aureus*, Optimization, Characterization.

Introduction

The global rise of antimicrobial resistance, particularly among Gram-positive pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), represents a significant public health challenge. MRSA is implicated in a broad spectrum of infections, ranging from superficial skin lesions to life-threatening systemic diseases, and its resistance to multiple classes of antibiotics significantly complicates treatment strategies.[1] The increasing prevalence of MRSA highlights the urgent need for alternative antimicrobial agents that are not only effective but also environmentally sustainable.[2] Nanotechnology is a rapidly advancing field with diverse applications in medicine, environmental science, renewable energy, and drug delivery.[3,4] Its contributions to drug encapsulation and targeted delivery have enhanced therapeutic efficacy while minimizing systemic toxicity.[5] Nanoparticles can be synthesized through chemical, physical, or biological methods. Although chemical synthesis allows for large-scale production, it often results in the generation of toxic by-products.[6] Physical methods, while effective, are typically energy-intensive and yield comparatively lower quantities of nanoparticles.[7]

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In contrast, biological or green synthesis offers a more sustainable alternative, being eco-friendly, cost-effective, and suitable for scalable applications.[8]Nanoparticle synthesis approaches are broadly categorized as either top-down or bottom-up. The top-down approach involves the breakdown of bulk materials into nanoscale structures. In contrast, the bottom-up approach assembles nanoparticles from individual atoms or molecules. Nanoparticle synthesis approaches are broadly categorized as either top-down or bottom-up. The top-down approach involves the breakdown of bulk materials into nanoscale structures, whereas the bottom-up approach assembles nanoparticles from individual atoms or molecules.[9] Chemical and biological synthesis methods typically employ the bottom-up strategy, while physical techniques generally align with the top-down approach. [10] Among bottom-up methods, green synthesis has gained attention for its minimal environmental impact, relying on biological sources such as enzymes, microbes, and plants.[11] Plant-mediated synthesis is particularly promising due to its rapid reaction rates, ease of handling, and the enhanced stability of the resulting nanoparticles.[12] Plants are rich in phytochemicals like flavonoids, tannins, polyphenols, terpenoids, and ascorbic acid that function as both reducing and capping agents during nanoparticle formation.[13] Compared to other green methods, plant-based approaches are more scalable, energy-efficient, and environmentally sustainable.[14] Silver nanoparticles (AgNPs) have been extensively studied for their excellent electrical conductivity, chemical stability, and wide range of biological activities, including antibacterial, antifungal, anticancer, antioxidant, antiviral, and anti-inflammatory effects.[15] The physicochemical characteristics of AgNPs, such as size, morphology, and surface charge, are influenced by various synthesis parameters, including pH, temperature, silver ion concentration, and the nature of reducing and capping agents.[16]

Acalypha godseffiana, an ornamental and medicinal plant, is rich in bioactive compounds such as flavonoids, alkaloids, polyphenols, and tannins, which contribute to its antioxidant, antimicrobial, and anti-inflammatory properties.[17] These phytochemicals make *A. godseffiana* a promising candidate for the green synthesis of silver nanoparticles (AgNPs), serving dual functions as both reducing and capping agents. Although numerous studies have investigated plant-mediated synthesis of AgNPs, there is limited research on the systematic optimization of synthesis parameters using *A. godseffiana* extract. The present study aims to synthesize, optimize, characterize, and evaluate the antibacterial efficacy of AgNPs synthesized using *A. godseffiana* leaf extract. Key synthesis parameters, including pH, temperature, reaction time, extract-to-silver nitrate ($AgNO_3$) volume ratio, and $AgNO_3$ concentration, will be systematically varied to determine optimal conditions. The resulting nanoparticles will be characterized using a comprehensive suite of analytical techniques: UV–Visible spectrophotometry, Scanning Electron Microscopy (SEM), Energy-Dispersive X-ray (EDX) analysis, Fourier-Transform Infrared (FTIR) spectroscopy, Zeta potential analysis, Dynamic Light Scattering (DLS), Thermogravimetric Analysis (TGA), and X-ray Diffraction (XRD), to evaluate their optical, structural, and morphological properties.

Furthermore, the antibacterial activity of the synthesized silver nanoparticles was evaluated against methicillin-resistant *Staphylococcus aureus* (MRSA) using minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays. To the best of our knowledge, this is the first report to demonstrate the antimicrobial efficacy of green-synthesized AgNPs derived from *Acalypha godseffiana* against MRSA. This study contributes not only to the growing body of research in green nanotechnology but also introduces a novel plant-based approach for combating antimicrobial resistance associated with multidrug-resistant pathogens.

Materials and Methods

Analytical grade silver nitrate and Methanol were purchased from Ceman Scientific LTD, Nigeria. All aqueous solutions were prepared using double-deionized distilled water.

Collection, Identification, and Extraction of Acalypha Godseffiana Leaves

Fresh, healthy leaves of *Acalypha godseffiana* were collected on 10th September 2023 from the botanical garden of Bayero University, Kano, Nigeria (approximately 11.9837° N, 8.4776° E). The plant was identified and authenticated by Dr. Baha'uddeen Said Adam, Department of Plant Biology, Bayero University Kano, and a herbarium accession number (BUKHAN 0352) was assigned. The leaves were thoroughly washed first with tap water and then with distilled water to remove dust and visible impurities. They were then cut into small pieces and air-dried at room temperature until brittle. The dried leaves were ground into a fine powder using an electrically driven mechanical blender. A 62.5 g portion of the powdered sample was subjected to Soxhlet extraction with 250 mL of Methanol for 3 hours. The resulting extract was concentrated using a rotary evaporator at 40 °C. The concentrated extract was poured into a Petri dish to a thickness of 4–5 mm and dried in an oven at 50 °C for 18 hours. The dried extract was subsequently stored in a refrigerator at −4 °C until further use. [18]

Green Synthesis of Silver Nanoparticles

Silver nanoparticles (AgNPs) were synthesized using *Acalypha godseffiana* leaf extract following a green synthesis protocol. A 10^{-3} M solution of silver nitrate ($AgNO_3$) was prepared by dissolving the salt in 90 mL of deionized water in a 250 mL reaction vessel. Subsequently, 10 mL of the plant extract solution (1 mg/mL) was added dropwise to the silver nitrate solution under continuous stirring at 1200 rpm using a magnetic stirrer. The reaction was allowed to proceed at room temperature for 1 hour. Following incubation, the mixture was centrifuged at 6000 rpm for 20 minutes, and this process was repeated three times. After each centrifugation, the resulting pellets were washed with deionized water to remove unbound or residual biomolecules. The final black solid residue was collected, dried, and stored in a clean container for further characterization.[19]

Optimization of various parameters for silver nanoparticle synthesis

The effects of six operational parameters, silver nitrate concentration, plant extract concentration, contact time, volume ratio, pH, and temperature, on the formation of silver nanoparticles (AgNPs) were systematically investigated. The synthesis process was monitored using a UV-Visible double-beam spectrophotometer. All experiments were conducted under batch conditions. The concentration of silver nitrate ($AgNO_3$) was varied between 0.002 M and 0.01 M, while the concentration of *A. godseffiana* methanolic leaf extract ranged from 1 mg to 5 mg. The Effect of contact time was studied by monitoring the reaction at intervals from 10 to 90 minutes. To assess the influence of volume ratio, different ratios of $AgNO_3$ solution to plant extract were tested. Additionally, the Effect of pH on nanoparticle synthesis was evaluated by adjusting the reaction mixture from pH 3 to 11. Temperature effects were assessed at five distinct levels: 45 °C, 60 °C, 75 °C, 90 °C, and 105 °C. In each case, the parameter of interest was varied while all other factors were held constant to isolate its influence on AgNP formation[20]

Characterization of green-synthesized silver nanoparticles

Visual inspection

The reduction of metal ions was visually inspected for color change in the reaction medium of AgNPs.

UV-Vis spectroscopy

The synthesized silver nanoparticles derived from Acalypha godseffiana were characterized using a UV-Vis double beam spectrophotometer (LI-2800 Ex, Lambda Scientific, China) over a wavelength range of 200–800 nm at multiple time intervals, with distilled water employed as the blank. [21]

Zeta analyzer

The size distribution and surface charge (zeta potential) of AgNPs were measured using a Zetasizer Nano ZS (Model: Nano ZS, Malvern Instruments Ltd., Malvern, UK) via dynamic light scattering (DLS). Samples were diluted with phosphate-buffered saline (PBS) (0.15 M, pH 7.2) before analysis, with a scattering angle of 90° maintained for particle size distribution measurements. [22]

2.4.3 Scanning electron microscopy

The particle size and microstructure of the silver nanoparticles (AgNPs) were examined using high-resolution scanning electron microscopy (SEM) (Model: JSM-7600F, JEOL Ltd., Tokyo, Japan). The AgNPs were dispersed in deionized water at a concentration of 1 mg/mL, homogenized using a sonication bath, and a drop of the resulting suspension was deposited onto a glass substrate, air-dried, and subsequently gold-coated before imaging. SEM analysis was complemented by energy-dispersive X-ray (EDX) spectroscopy to confirm the elemental composition of the nanoparticles.[23]

Fourier-transform infrared (FTIR) spectroscopy

Fourier-transform infrared (FTIR) spectroscopy was used to identify functional groups involved in nanoparticle synthesis, using an FTIR spectrometer (Model: IR Affinity-1S, Shimadzu Corporation, Kyoto, Japan). AgNPs samples were mixed with potassium bromide (KBr) in a 1:100 ratio, finely ground, and compressed into a pellet. The spectra were recorded in the range of 400-4000 cm^{-1} at a resolution of 4 cm^{-1}. [24]

X-ray diffraction (XRD)

The crystalline structure and phase composition of the silver nanoparticles (AgNPs) were characterized using an X-ray diffractometer (Model: X'Pert PRO, PANalytical B.V., Almelo, The Netherlands). The AgNP suspension was deposited onto a glass slide and dried on a hot plate at 50 °C. XRD analysis was conducted using Cu-Kα radiation (λ = 0.154187 nm) operated at 30 kV and 20 mA. Data

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were collected over a 2θ range of 30°–80° at a scan rate of 0.03°/s. Phase identification was performed using X'Pert HighScore software.[25]

Thermo gravimetric analysis (TGA)
Thermogravimetric analysis (TGA) was conducted to assess the thermal stability of the synthesized silver nanoparticles (AgNPs) using a TA Instruments Q Series™ DSC/TGA system (Model Q600, TA Instruments, New Castle, DE, USA). Approximately 5 mg of the sample was placed in a platinum crucible and heated from room temperature to 800 °C at a constant rate of 10 °C/min under a nitrogen atmosphere with a flow rate of 50 mL/min.[26]

Antibacterial Activity of the Nanoparticles
The antibacterial activity of *Acalypha godseffiana*-mediated silver nanoparticles (AgNPs) against methicillin-resistant *Staphylococcus aureus* (MRSA) was evaluated using the disc diffusion method. A standard MRSA strain (BAA-1720), obtained from the American Type Culture Collection (ATCC), was sourced from the Microbiology Laboratory, Bayero University, Kano. Sterile paper discs (6 mm diameter) were impregnated with the test solutions and placed on Mueller-Hinton agar (MHA) plates previously inoculated with 30 µL of an MRSA suspension (4×10^5 CFU/mL). Ampicillin and fusidic acid were used as the negative and positive controls, respectively. The zone of inhibition (ZOI) around the discs was measured in millimeters for silver nitrate ($AgNO_3$), plant extract, and AgNPs. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the AgNPs were determined following standard procedures. Briefly, the green-synthesized AgNPs were serially diluted to concentrations of 5000, 2500, 1250, 625, 312, and 156 µg/mL. Equal volumes of each concentration were added to MRSA-inoculated Mueller-Hinton broth (MHB) at a final bacterial concentration of 10^8 CFU/mL and incubated at 37 °C for 24 hours. The MIC was defined as the lowest concentration at which no visible bacterial growth was observed. To determine the MBC, 30 µL aliquots from tubes showing no visible growth were plated onto MHA and incubated overnight at 37 °C. The MBC was recorded as the lowest concentration that yielded no bacterial colonies on the agar plates.[27]

Results and Discussion

Green synthesis of silver nanoparticles
A distinct color change in the reaction mixture evidenced the initial indication of silver nanoparticle (AgNP) synthesis. Upon the addition of *Acalypha godseffiana* leaf extract to a one mM silver nitrate ($AgNO_3$) solution, the solution's appearance changed from colorless to golden-brown (Fig. 1A). This visual transformation suggests the reduction of silver ions (Ag^+) to elemental silver (Ag^0), leading to the formation of silver nanoparticles. Such color changes are widely reported in green synthesis approaches, wherein plant extracts serve as natural reducing agents to facilitate metal ion reduction. The development of the characteristic brown color is attributed to the excitation of surface plasmon resonance (SPR) in the silver nanoparticles. It serves as an initial qualitative confirmation of nanoparticle formation.[28]

Visual/UV-Vis spectral analysis
To further verify the synthesis of silver nanoparticles, UV-Visible spectroscopy was performed. The analysis revealed a distinct absorption peak at approximately 432 nm (Fig. 1B), characteristic of the surface plasmon resonance (SPR) phenomenon exhibited by silver nanoparticles. This SPR peak results from the collective oscillation of conduction electrons at the nanoparticle surface when excited by incident light, serving as a definitive marker of nanoparticle formation. Moreover, the position and sharpness of the SPR peak provide valuable information regarding the nanoparticle size and dispersion, with a sharp peak indicating the presence of small, well-dispersed particles. These spectroscopic results corroborate the observed color change and confirm the successful green synthesis of silver nanoparticles using *Acalypha godseffiana* extract.[29]

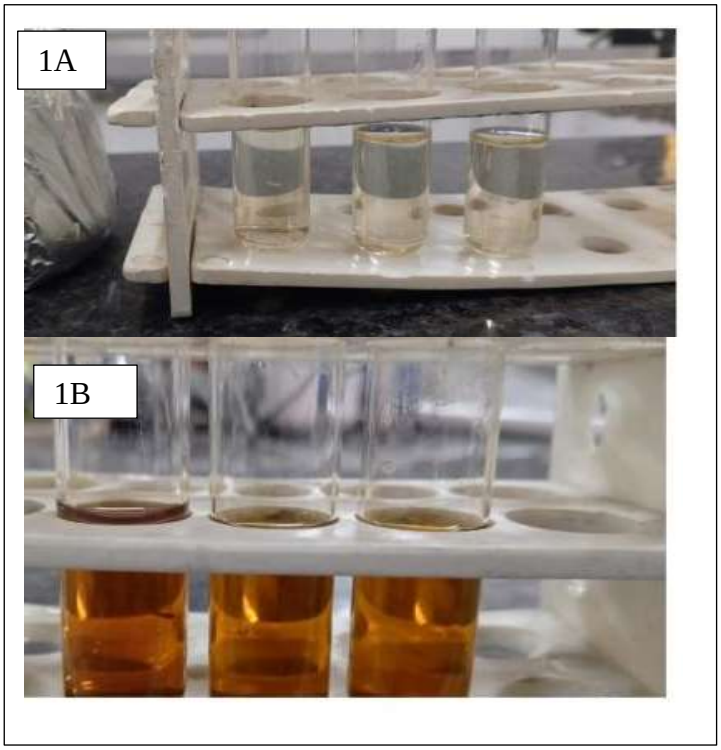

Figure 1: (A) Visual inspection of color change as AgNPs are synthesized, (B) UV–Visible spectra of AgNPs

Optimization of conditions for the synthesis of silver nanoparticles
Effect of pH.
pH is a critical factor influencing the synthesis, size, stability, and optical properties of silver nanoparticles (AgNPs). In this study, the formation rate of AgNPs increased progressively with rising pH, reaching a peak at pH 11. Notably, at pH 9, the synthesized nanoparticles exhibited high stability, demonstrated by a sharp absorbance peak at 447 nm (Fig. 2A), leading to their selection as the optimum pH for nanoparticle synthesis using *Acalypha godseffiana* leaf extract.[30] The enhanced nanoparticle formation under alkaline conditions is attributed to the increased availability of deprotonated functional groups, such as phenolic and hydroxyl moieties, which act as effective reducing and stabilizing agents.[31] The distinct absorbance peak at 447 nm further confirms efficient reduction and uniform nanoparticle distribution at this pH. Moreover, the observed stability suggests strong electrostatic repulsion between particles, preventing aggregation. These findings align with previous reports indicating that higher pH values promote faster nucleation and improved control over nanoparticle size in green synthesis.[32]

Effect of Time
Reaction time plays a crucial role in the synthesis of silver nanoparticles (AgNPs) using *Acalypha godseffiana* leaf extract. As the reaction proceeded, a gradual color change from colorless to golden brown was observed, indicating the reduction of silver ions and the formation of AgNPs. UV-Visible spectroscopic analysis showed a progressive increase in the surface plasmon resonance (SPR) peak intensity over time, with a prominent peak at 430 nm observed at 90 minutes (Fig. 2B). This increase in peak intensity corresponds to enhanced nanoparticle formation and stability. The absorbance of the SPR peak is a function of contact time, increasing with longer reaction durations. Maximum absorbance was recorded at 90 minutes, which was therefore considered the optimal reaction time for AgNP synthesis. These findings are consistent with earlier reports indicating that prolonged reaction times facilitate continuous nucleation and growth of nanoparticles, resulting in higher yield and improved optical properties.[33]

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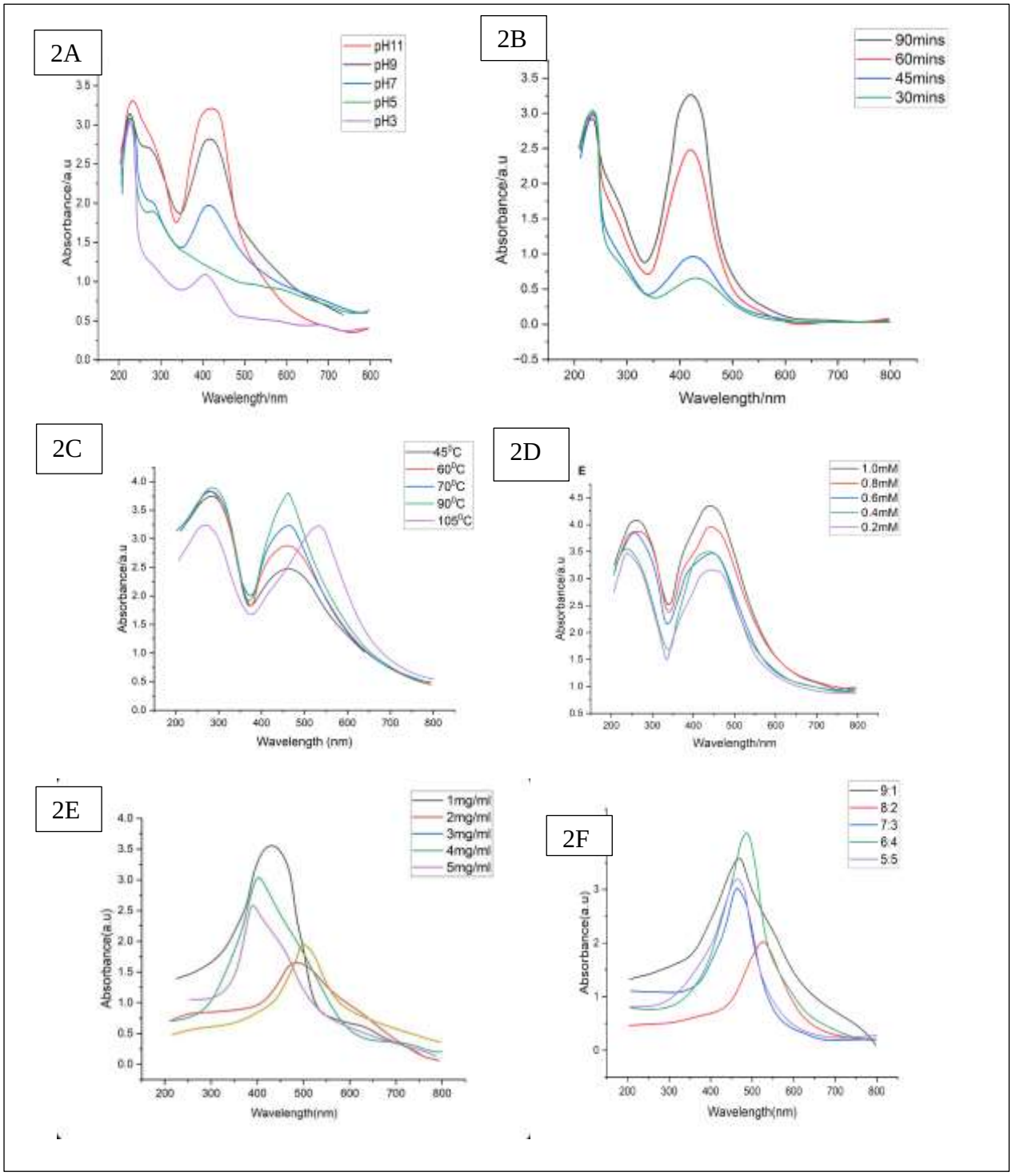

Figure 2: Effect of various parameters on synthesis of AgNPs (A) pH, (B) Time, (C) Temperature, (D) Concentration of $AgNO_3$, (E) Concentration of Plant extract, (F) Silver nitrate to plant ratio

Effect of Temperature

Temperature significantly influences both the kinetics of silver ion reduction and the physicochemical characteristics of the synthesized nanoparticles. An increase in absorbance and solution color intensity was observed with rising temperature, indicating enhanced nanoparticle formation up to a certain point. Optimal synthesis occurred at 90 °C, where a sharp SPR peak was detected at 431 nm (Fig. 2C), suggesting the production of smaller, well-dispersed nanoparticles. At temperatures beyond this point, a decline in absorbance and nanoparticle stability was noted. The improved nanoparticle synthesis at elevated temperatures is attributed to the accelerated reduction of Ag^+ ions by phytochemicals in the *A. godseffiana* extract, promoting rapid nucleation and limiting aggregation. These results corroborate previous studies where higher temperatures enhanced reaction kinetics and

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particle uniformity.[34] Accordingly, 90 °C was selected as the optimal temperature for AgNP synthesis using *A. godseffiana*.

Effect of silver nitrate concentration

The concentration of silver nitrate also had a marked impact on AgNP synthesis. Increasing $AgNO_3$ concentrations led to a rise in both SPR peak absorbance and wavelength, indicating greater nanoparticle yield and improved stability. Among the tested concentrations, one mM was identified as optimal, exhibiting maximum absorbance at 447 nm (Fig. 2D). Although the absorbance initially increased up to 0.6 mM, a subsequent decline was observed at 0.8 mM and above, accompanied by a reduction in peak wavelength and nanoparticle stability. These suggest that excessively high precursor concentrations may lead to particle agglomeration or suboptimal reduction efficiency. These results are consistent with earlier findings that optimal silver ion concentration is critical for controlling particle size, uniformity, and colloidal stability during green synthesis.[35]

Effect of plant extract concentration

The concentration of *Acalypha godseffiana* extract significantly influenced the synthesis and characteristics of silver nanoparticles. An increase in extract concentration was associated with a corresponding rise in both particle size and spectral intensity. However, the optimal extract concentration was identified as 1 mg/mL, at which the synthesized nanoparticles exhibited a sharp surface plasmon resonance (SPR) peak at 447 nm (Fig. 2E), indicating the formation of small, stable, and well-dispersed particles. These findings suggest that lower extract concentrations favor more uniform nanoparticle formation, likely due to controlled reduction and capping by bioactive compounds[36]

Effect of volume ratio of silver nitrate to plant extract

The volume ratio of silver nitrate to plant extract also had a pronounced impact on nanoparticle synthesis. Variations in the $AgNO_3$:*A: A. Godseffiana* extract ratio altered the size, shape, and distribution of the resulting AgNPs. As the proportion of plant extract increased, both color intensity and absorbance values decreased. This is likely due to the formation of polydispersed nanoparticles at higher extract volumes, resulting in peak broadening and shifts in the SPR band. The optimal silver nitrate-to-extract ratio produced a well-defined SPR peak at 441 nm (Fig. 2F), indicating the best balance between reduction and stabilization processes[37]

Characterization of Synthesized AgNPs

Zeta potential

Zeta potential analysis revealed that the silver nanoparticles synthesized using *A. godseffiana* extract carried a strong negative surface charge, indicating excellent colloidal stability. While the instrument-reported average zeta potential was –14.6 mV, detailed distribution analysis showed that the dominant nanoparticle population had a potential of –57.5 mV (Fig. 3A). This value exceeds the ±30 mV threshold typically associated with stable nanoparticle suspensions.[38] The high negative charge promotes electrostatic repulsion between particles, minimizing aggregation and supporting uniform dispersion. Such stability is advantageous in biomedical and catalytic applications, where aggregation could hinder nanoparticle efficacy.[39] Minor populations at -14.6 mV and -3.23 mV reflect slight heterogeneity, but they do not significantly detract from the overall colloidal integrity.

Dynamic Light Scattering (DLS) Analysis

Dynamic light scattering (DLS) analysis revealed a multimodal size distribution among the synthesized AgNPs. A dominant peak corresponding to particles in the 30–50 nm range was observed (Fig. 3B), representing the primary nanoscale fraction. This size range aligns with the expected morphology of AgNPs and is suitable for applications in antimicrobial and drug delivery systems. Larger peaks in the DLS spectrum are attributed to agglomerates, which are common in plant-mediated synthesis and do not compromise the integrity or functionality of the core nanoparticles.[40,41] The 30–50 nm population also corresponds well with findings from UV-Vis and XRD analyses, confirming consistency across characterization techniques.

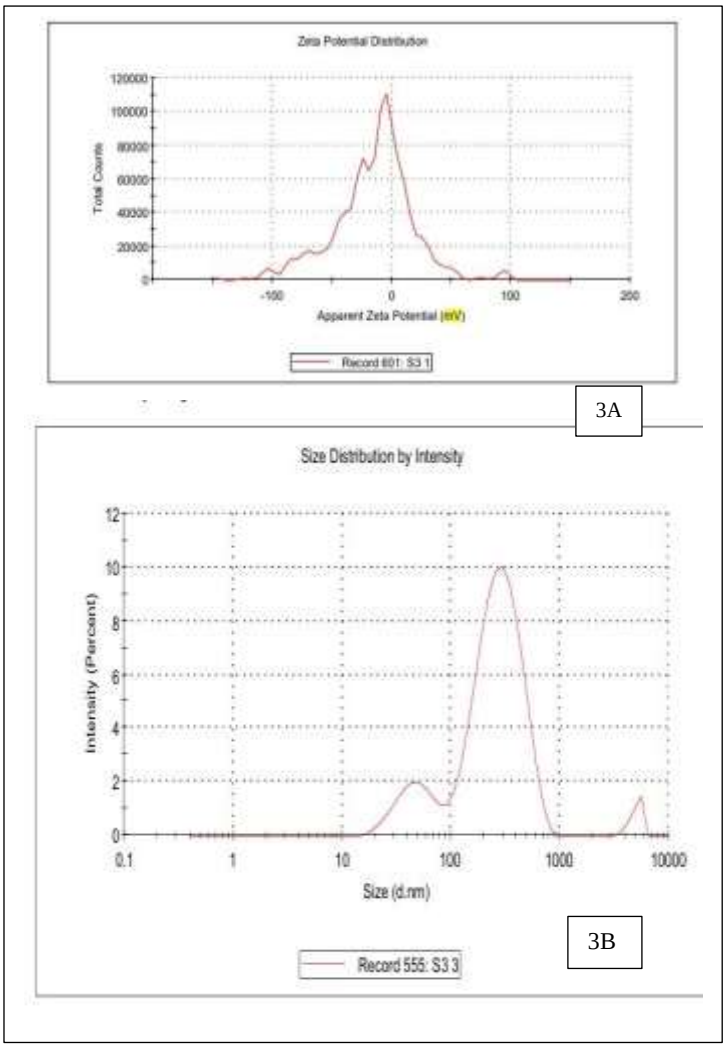

Figure 3: (A) Zeta potential of AgNPs (3B) DLS of AgNPs

Scanning Electron Microscopy (SEM) Analysis

SEM imaging revealed heterogeneous surface morphology, with irregular and aggregated particles distributed across a rough matrix (Fig. 4A). Although AgNPs are often spherical, the synthesized particles exhibited substantial agglomeration, likely due to phytochemicals from *A. godseffiana* acting as natural capping agents. Some rounded nanoparticle features were visible; however, their fused appearance suggests limited dispersion and possible structural deformation. [42] These morphological traits reflect the partially crystalline nature of green-synthesized AgNPs and are consistent with particle coalescence mediated by biological stabilizers.

Energy Dispersive X-ray (EDX) Analysis

The energy-dispersive X-ray (EDX) spectrum of the silver nanoparticles synthesized using *A. godseffiana* extract confirmed the presence of elemental silver, with a prominent peak at 3.0–3.2 keV (Fig. 4B), indicative of successful nanoparticle formation. In addition to silver, other elements detected—such as C, O, P, S, K, Ca, and Cl are attributed to phytochemicals in the plant extract. These components likely functioned as natural reducing and stabilizing agents, supporting effective biological capping of the nanoparticles. The presence of chlorine further suggests the minor formation of AgCl, corroborating the XRD findings.[43] Collectively, the EDX results reinforce the green synthesis mechanism by confirming both nanoparticle formation and

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the involvement of plant-derived biomolecules in nanoparticle stabilization.

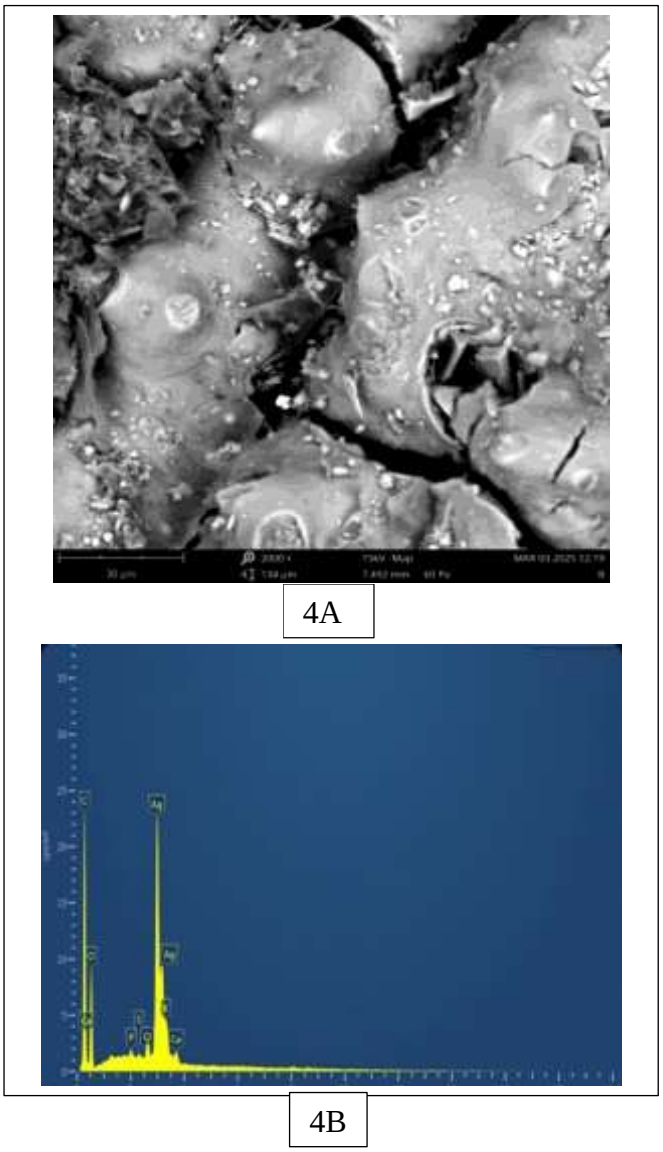

Figure 4: (A) SEM image of AgNPs; (B) EDX of AgNPs

Fourier-Transform Infrared (FTIR) Analysis

The FTIR spectrum of *A. godseffiana* leaf extract exhibited strong absorption bands at 3325 cm^{-1} (O–H stretching), 2922 cm^{-1} (C–H stretching), 1634 cm^{-1} (C=O stretching), and 1035 cm^{-1} (C–O stretching) (Fig. 5A), indicating the presence of phenolics, flavonoids, and other bioactive compounds.[44] Following nanoparticle synthesis, the O–H peak at 3325 cm^{-1} showed reduced intensity, and the C=O peak at 1634 cm^{-1} shifted (Fig. 5B), suggesting interaction with Ag^{+} ions. Additionally, the emergence of new peaks at 407, 418, and 578 cm^{-1}, corresponding to Ag–O and Ag–N vibrations, confirmed the formation of AgNPs.[45] These spectral changes indicate that biomolecules present in *A. godseffiana* played key roles in both the reduction of silver ions and the stabilization of the resulting nanoparticles.

X-ray Diffractometry (XRD) Analysis

The X-ray diffraction (XRD) pattern of silver nanoparticles synthesized using *A. godseffiana* extract and silver nitrate displayed distinct diffraction peaks at 38.14°, 44.32°, and 64.51° (Fig. 6A), corresponding to the (111), (200), and (220) crystallographic planes of face-centered cubic (fcc) silver, in agreement with the JCPDS file No. 04–0783. Additional peaks at 32.32° and 34.39° were attributed to chlorargyrite (AgCl) and muscovite, respectively, likely originating from residual phytochemicals or plant-derived capping agents.[46,47] The average crystallite size, estimated using the Debye–Scherrer equation, was approximately 29.0 nm, confirming the formation of nanoscale particles.[48] The dominant (111) reflection suggests a preferred growth orientation, which is characteristic of silver nanoparticles. The presence of secondary phases further supports the role of bioactive compounds in the extract in nanoparticle stabilization. Overall, the sharp diffraction peaks indicate good crystallinity and practical green synthesis of AgNPs [53]

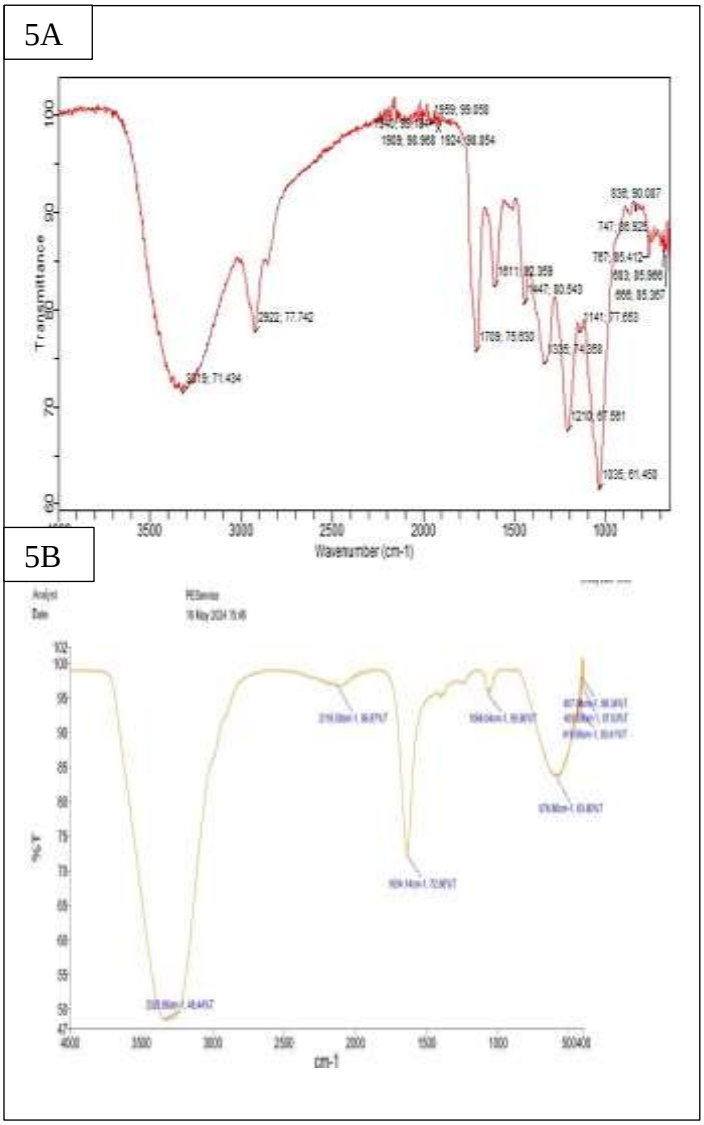

Figure 5: Fourier-transform infrared spectroscopy (FTIR) spectra of (A) *Acalypha Godseffiana* plant, (B) Synthesized silver nanoparticles

Thermo gravimetric Analysis (TGA)

The thermal stability of the biosynthesized silver nanoparticles was evaluated using thermogravimetric analysis (TGA). The TGA curve revealed that the predominant weight loss occurred between 350 °C and 450 °C (Fig. 6B), indicating the decomposition of organic constituents,

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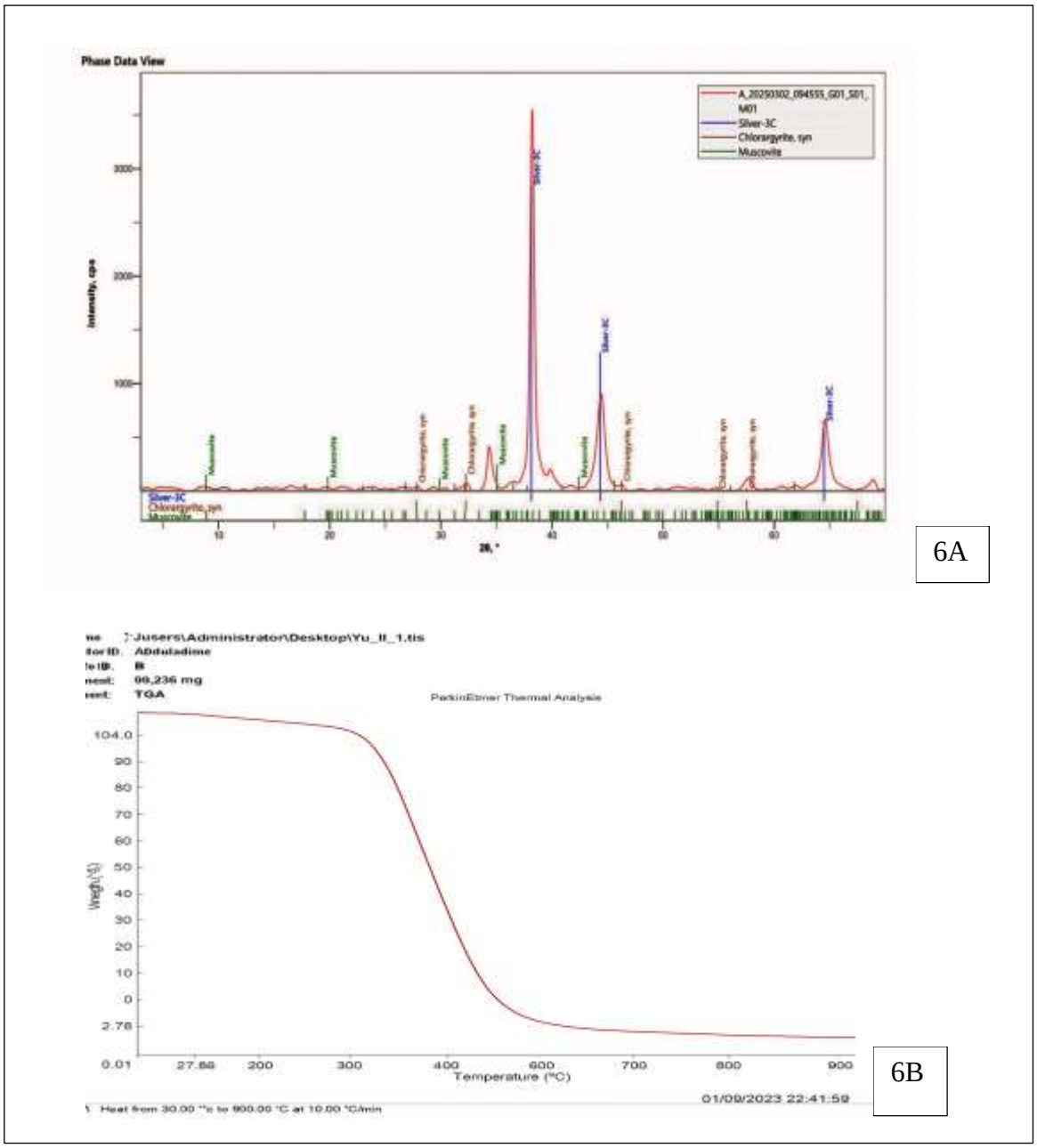

Figure 6: (A) XRD spectrum of AgNPs; (B) TGA curve of AgNPs

primarily phytochemicals from the *A. godseffiana* extract that acted as capping and stabilizing agents.[50] Minimal weight loss was observed below 350 °C and beyond 450 °C, suggesting that the majority of thermally labile compounds degraded within this intermediate temperature range. The stable residual mass above 450 °C corresponds to the metallic silver core, confirming the formation of thermally stable nanoparticles. This thermal profile demonstrates the robust thermal stability of the synthesized AgNPs and underscores the critical role of *A. godseffiana* phytochemicals in nanoparticle stabilization during biosynthesis.[51]

Antimicrobial study against methicillin-resistant Staphylococcus aureus *(MRSA)*

The antibacterial efficacy of *A. godseffiana*-synthesized silver nanoparticles (AgNPs) against methicillin-resistant *Staphylococcus aureus* (MRSA) was assessed using the disc diffusion method. Ampicillin (10 µg) and fusidic acid (10 µg) were used as negative and positive controls, respectively. The zones of inhibition (ZOI) for silver nitrate, *A. godseffiana* extract, and the synthesized AgNPs were 7.2 mm, 10.33 mm, and 20.78 mm, respectively, demonstrating a substantial enhancement in antibacterial activity by the nanoparticles. These findings underscore the potent anti-MRSA potential of the biosynthesized AgNPs, which was further supported by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assessments (Table 1).[52] The AgNPs exhibited a MIC of 625 µg/mL and an MBC of 1250 µg/mL against MRSA. These results align with previous studies reporting strong antimicrobial performance of green-synthesized AgNPs, thereby validating the effectiveness of *A. godseffiana* as a promising bio-reducing and stabilizing agent for silver nanoparticle synthesis. [53]

Table 1: MIC and MBC values of $AgNO_3$, plant extract, and AgNPs of *A. godseffiana*

Test samples	MIC(µg/ml)	MBC(µg/ml)
$AgNO_3$	12500	25000
Plant extract	2500	5000
AgNPs of *A. godseffiana*	625	1250

Conclusion

In this study, silver nanoparticles (AgNPs) were successfully synthesized using *Acalypha godseffiana* leaf extract, which served as a natural reducing and stabilizing agent. Nanoparticle formation was visually indicated by a color change from colorless to golden brown and confirmed by a UV-Vis absorption peak at 432 nm, corresponding to surface plasmon resonance (SPR). XRD analysis revealed crystalline nanoparticles predominantly oriented along the (111) plane, with an average crystallite size of approximately 29 nm. FTIR spectra confirmed the involvement of phytochemicals in both reduction and stabilization processes. SEM imaging showed irregular and somewhat aggregated particle morphologies, while dynamic light scattering (DLS) analysis identified a dominant particle population in the 30–50 nm range. The zeta potential value of –57.5 mV indicated strong colloidal stability. Thermal stability of the synthesized AgNPs was demonstrated by TGA analysis, with primary decomposition occurring between 350°C and 450°C. Optimization studies revealed that the most effective synthesis conditions were a pH of 9, temperature of 90 °C, one mM silver nitrate concentration, 90-minute reaction time, 1 mg/mL plant extract concentration, and a 1:9 extract-to-silver nitrate volume ratio. These optimized parameters facilitated the efficient and reproducible green synthesis of stable AgNPs. Moreover, the biosynthesized AgNPs exhibited significant antimicrobial activity against MRSA, with concentration-dependent inhibition observed. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were both found to be 31.25 µg/mL, highlighting the potent bactericidal capability of the nanoparticles at relatively low concentrations. In conclusion, this study demonstrates the feasibility of utilizing *A. godseffiana* for eco-friendly synthesis of silver nanoparticles with promising physicochemical properties and vigorous antibacterial activity against MRSA. These findings underscore the potential of green-synthesized AgNPs for application in biomedical and environmental settings. Further investigations into their mechanisms of action, biocompatibility, and clinical applicability are warranted to advance their development as alternative antimicrobial agents for combating multidrug-resistant infections.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that they will bear any liability for claims relating to the content of this article.

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References

1 Douglas EJA, Wulandari SW, Lovell SD, Laabei M. Novel antimicrobial strategies to treat multidrug-resistant *Staphylococcus aureus* infections. Microb Biotechnol. 2023; 16(7): 1456–1474.
2 Shariati A, Dadashi M, Chegini Z, Belkum A, Mirzaii M, Khoramrooz SS, Sarokhalil DD. The global prevalence of daptomycin, tigecycline, quinupristin/dalfopristin, and linezolid-resistant *Staphylococcus aureus* and coagulase-negative staphylococci strains: A systematic review and meta-analysis. Antimicrob Resist Infect Control. 2020; 9(1): 1–20.
3 Malik S, Kursunoglu B, Boakye-Yiadom K, Decho AW, Ozturk T, Bilgili H, Duman F, Mamillapalli R, Tekinay T, Guler MO. Emerging applications of nanotechnology in healthcare: diagnostics, disease treatment, regenerative medicine, gene therapy, and beyond. ACS Appl Bio Mater. 2023; 6(3): 1338-1371.
4 Nkosi NC, Basson AK, Ntombela ZG, Dlamini NG, Pullabhotla RVSR. Green synthesis and characterization of iron nanoparticles synthesized from bioflocculant for wastewater treatment: A review. Biotechnol Notes. 2025; 6: 10–31.
5 Patel RR, Singh SK, Singh M. Green synthesis of silver nanoparticles: Methods, biological applications, delivery and toxicity. Mater Adv. 2023; 4(8): 1831–1849.
6 Osman AI, Zhang Y, Farghali M, Rashwan AK, Eltaweil AS, Abd El Monaem EM, Mohamed IMA, Badr MM, Ihara I, Rooney DW, Yap P. Synthesis of green nanoparticles for energy, biomedical, environmental, agricultural, and food applications: A review. Environ Chem Lett. 2024; 22(2): 1089–1136.
7 Yadav RK, Jangeer S, Parashar R, Sharma G, Meena P, Meena MK, Patel DD. A critical review on nanoparticles synthesis: Physical, chemical, and biological approaches. Int J Creat Res Thoughts. 2023; 11(10): 834–848.
8 Al-radadi NS. Laboratory scale medicinal plants mediated green synthesis of biocompatible nanomaterials and their versatile biomedical applications. Saudi J Biol Sci. 2022; 29(5): 3848-3870.
9 Baig N, Kammakakam I, Falath W, Kammakakam I. Nanomaterials: A review of synthesis methods, properties, recent progress, and challenges. Mater Adv. 2021; 2(6): 1821–1871.
10 Alharbi NS, Alsubhi NS, Felimban AI. Green synthesis of silver nanoparticles using medicinal plants: Characterization and application. J Radiat Res Appl Sci. 2022; 15(3): 109–124.
11 Ijaz I, Gilani E, Nazir A, Bukhari A. Detail review on chemical, physical and green synthesis, classification, characterizations and applications of nanoparticles. Green Chem Lett Rev. 2020; 13(1): 59–81.
12 Pirsaheb M, Salimi A, Jafari A, Sillanpää M, Fakhri Y, Khaneghah AM. Green synthesis of nanomaterials by using plant extracts as reducing and capping agents. Environ Sci Pollut Res Int. 2024; 31(18): 24768–24787.
13 Shahzadi S, Fatima S, Ul Ain Q, Shafiq Z, Janjua MRSA. A review on green synthesis of silver nanoparticles (SNPs) using plant extracts: A multifaceted approach in photocatalysis, environmental remediation, and biomedicine. RSC Adv. 2025; 15(6): 3858–3903.
14 Labulo AH, David OA, Terna AD. Green synthesis and characterization of silver nanoparticles using *Morinda lucida* leaf extract and evaluation of its antioxidant and antimicrobial activity. Chem Pap. 2022; 76(12): 7313–7325.
15 Santos TS, Silva TM, Cardoso JC, Albuquerque-Júnior RLC, Zielinska A, Souto EB, Severino P, Mendonça MDC. Biosynthesis of silver nanoparticles mediated by nanopesticides and toxicity. Antibiotics. 2021; 10(7): 1–13.
16 Kaabipour S, Hemmati S. A review on the green and sustainable synthesis of silver nanoparticles and one-

dimensional silver nanostructures. Beilstein J Nanotechnol. 2021; 12(1): 102–136.
17 Asekunowo AK, Ashafa AOT, Okoh O, Asekun OT, Familoni OB. Polyphenolic constituents, antioxidant and hypoglycaemic potential of leaf extracts of *Acalypha godseffiana* from Eastern Nigeria: In vitro study. J Med Plants Econ Dev. 2019; 3(1): 1–9.
18 Mokaizh AAB, Gharib FA, Al-Yahyai R, Al-Saidi B, Al-Lawati Y, Al-Barwani HM. Extraction and characterization of biologically active components from *Commiphora gileadensis* leaves. Processes. 2024; 12(8): 1567–1585.
19 Kavaz D, Umar H, Shehu S. Synthesis, characterization, antimicrobial and antimetastatic activity of silver nanoparticles synthesized from *Ficus ingens* leaf. Artif Cells Nanomed Biotechnol. 2019; 46(S3): S1193–S1203
20 Olubodun SO, Eriyamremu GE, Ayevbuomwan ME, Nzoputa CI. In vitro antioxidant activity and phytochemical analyses of *Acalypha godseffiana* (Euphorbiaceae) leaf extracts. Niger J Nat Prod Med. 2021; 25(1): 75–79.
21 Khanal LN, Sharma KR, Paudyal H, Parajuli K, Dahal B, Ganga G, Pokharel YR, Kalauni SK. Green synthesis of silver nanoparticles from root extracts of *Rubus ellipticus* Sm. and comparison of antioxidant and antibacterial activity. J Nanomater. 2022; 2022: 1–11.
22 Karuppannan P, Saravanan K, Ashokkumar M, Egbuna C. Preparation of bio-synthesized Ag nanoparticles and assessment of their antidiabetic and antioxidant potential against STZ-induced diabetic albino rats. J Biomater Sci Polym Ed. 2024; 35(5): 535–558.
23 Bernardes LMM, Malta SM, Santos ACC, Silva RA, Rodrigues TS, Silva MNT, Bittar VP, Borges ALS, Justino AB, Nossol ABS, Martins MM, Espíndola FS, M-Silva AP, U-Vieira C. Green synthesis, characterization, and antimicrobial activity of silver nanoparticles from water-soluble fractions of Brazilian kefir. Sci Rep. 2025; 15(1): 1–18.
24 Hawar SN, Al-shmgani HS, Al-kubaisi ZA, Sulaiman GM, Dewir YH, Rikisahedew JJ. Green synthesis of silver nanoparticles from *Alhagi graecorum* leaf extract and evaluation of their cytotoxicity and antifungal activity. J Nanomater. 2022; 2022: 1–8.
25 Panda MK, Dhal NK, Kumar M, Mishra PM, Behera RK. Green synthesis of silver nanoparticles and their potential effect on phytopathogens. Mater Today Proc. 2021; 35(2): 233–238.
26 Moteriya P, Chanda S. Biosynthesis of silver nanoparticles formation from *Caesalpinia pulcherrima* stem metabolites and their broad spectrum biological activities. J Genet Eng Biotechnol. 2018; 16(1): 105–113.
27 Parvekar P, Palaskar J, Metgud S, Maria R, Dutta S. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of silver nanoparticles against *Staphylococcus aureus*. Biomater Investig Dent. 2020; 7(1): 105–109.
28 Sulthana RN, Rajanikanth A. Green synthesis of silver nanoparticles using seed extract of *Foeniculum vulgare* and their antibacterial activity. Int J Curr Res Biosci Plant Biol. 2018; 5(7): 77–83.
29 Mfon RE, Deshi JJ, Al Amri Z, Madugu JS. Biochemical synthesis, characterization, and electrodeposition of silver nanoparticles on a gold substrate. J Niger Soc Phys Sci. 2022; 4(3): 1–6.
30 Mardiyanto M, Apriani EF, Heylken FP. The role of temperature and pH in the synthesis of silver nanoparticles using *Areca catechu* L. seed extract as bioreductor. Farmacia. 2023; 71(2): 244–253.
31 Utami YP, Yulianty R, Djabir YY, Alam G. Green synthesis, from some medicinal plants. BMC Biotechnol. 2024; 24(1): 1–22.
46 Hlapisi N, Songca SP, Ajibade PA. Morphological and structural properties of silver/chlorargyrite nanoparticles optimization and characterization of carrot extract silver nanoparticles. Trop J Nat Prod Res. 2023; 8(12): 5955–5961.
32 Wang Y, Wei S. Green fabrication of bioactive silver nanoparticles using *Mentha pulegium* extract under alkaline: an enhanced anticancer activity. ACS Omega. 2022; 7(2): 1494–1504.
33 Kumar I, Gangwar C, Yaseen B, Pandey PK, Mishra SK, Naik RM. Kinetic and mechanistic studies of the formation of silver nanoparticles by nicotinamide as a reducing agent. ACS Omega. 2022; 7(16): 13778–13788.
34 Aliero AS, Hammawa H, Usman AS, Aliero MS, Raji AH, Umar IA, Alhassan AM, Aliero AA. Harnessing palm oil mill effluent for the green synthesis of silver nanoparticles: optimization via response surface methodology and assessment of antibacterial activity. J Inorg Organomet Polym Mater. 2025; 35(9): 3580–3596.
35 Mishra AK, Tiwari KN, Saini R, Kumar P, Mishra SK, Yadav VB, Nath G. Green synthesis of silver nanoparticles from leaf extract of *Nyctanthes arbor-tristis* L. and assessment of its antioxidant, antimicrobial response. J Inorg Organomet Polym Mater. 2020; 30(6): 2266–2278.
36 Sneha KD, Sarika SP, Tejonmayi S, Phatake YB, Sneha GM, Shivanjali SD, Marathe RJ, Deshmukh RB, Dharmadhikari SM. Green synthesis of silver nanoparticles by using stem, leaves, and fruits extracts of umber (*Ficus racemosa*). Int J Pharm Investig. 2020; 10(3): 312–319.
37 Soshnikova V, Kim YJ, Singh P, Huo Y, Markus J, Ahn S, Castro-Aceituno V, Mathiyalagan R, Yang DC. Cardamom fruits as a green resource for facile synthesis of gold and silver nanoparticles and their biological applications. Artif Cells Nanomed Biotechnol. 2018; 46(1): 108–117.
38 Erdogan O, Abbak M, Demirbolat GM, Birtekocak F, Aksakal O, Pasa S. Green synthesis of silver nanoparticles via *Cynara scolymus* leaf extracts: The characterization, anticancer potential with photodynamic therapy in MCF7 cells. PLoS One. 2019; 14(6): 1–15.
39 Jeon YN, Ryu SJ, Lee HY, Kim JO, Baek JS. Green synthesis of silver nanoparticles using black mulberry and characterization, phytochemical, and bioactivity. Antibiotics. 2024; 13(8): 1–14.
40 Vanlalveni C, Lallianrawna S, Biswas A, Selvaraj M, Changmai B, Rokhum SL. Green synthesis of silver nanoparticles using plant extracts and their antimicrobial activities: A review of recent literature. RSC Adv. 2021; 11(5): 2804–2837.
41 Velidandi A, Dahariya S, Pabbathi NPP, Kalivarathan D, Baadhe RR. A review on synthesis, applications, toxicity, risk assessment, and limitations of plant extracts for synthesizing silver nanoparticles. NanoWorld J. 2020; 6(3): 35–60.
42 Keskin Seyhan Aslan C, Baran MF, Baran A, Eftekhari A, Adıcan MT, Ahmadian E, Arslan S, Mohamed AJ. Green synthesis and characterization of silver nanoparticles using *Anchusa officinalis*: Antimicrobial and cytotoxic potential. Int J Nanomedicine. 2025; 20(4): 4481–4502.
43 Faisal NH, Obaid NA, Ali NW, Al-Shawi AA, Ibrahim RG, Hassan AN, Kadhum AJ, Mohsein OA, Luaibi IN. Green synthesis of silver nanoparticles using the seeds of *Lupinus luteus*: characterization and assessment of the antibacterial and anticancer activities. Asian Pac J Cancer Prev. 2025; 26(9): 3405-3414.
44 Khalil MMH, Ismail EH, El-Baghdady KZ, Mohamed D. Green synthesis of silver nanoparticles using olive leaf extract and its antibacterial activity. Arab J Chem. 2014; 7(6): 1131–1139.
45 Asefian S, Ghavam M. Green and environmentally friendly synthesis of silver nanoparticles with antibacterial properties

prepared using *Senecio madagascariensis* leaf extract and interaction studies with bovine serum albumin. MRS Adv. 2024; 9(10): 830–836.
47 Halilu EM, Ngweh VA, Airemwen CO. Green synthesis of

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silver nanoparticles from *Parinari curatellifolia* methanol stem bark extract and evaluation of antioxidant and antimicrobial activities. Trop J Nat Prod Res. 2023; 7(3): 2498–2505.

48 Castañeda-Aude JE, Morones-Ramírez JR, De Haro-Del Río DA, León-Buitimea A, Barriga-Castro ED, Escárcega-González CE. Ultra-small silver nanoparticles: A sustainable green synthesis approach for antibacterial activity. Antibiotics. 2023; 12(3): 1–13.

49 Jamila T, Lucky E, Eli D. Preparation and characterization of high-performance dye-sensitized solar cells with silver nanoparticles in nanocomposite photoanode. J Niger Soc Phys Sci. 2020; 2(1): 7–11.

50 Chandrasekaran M, Chinnaiyan U, Sivaprakasam S, Paramasivam S. Biogenic synthesis and characterization of silver nanoparticles using a combined leaf extract for antibacterial and biofilm inhibition properties. Trop J Nat Prod Res. 2025; 9(3): 1089–1096.

51 Labulo AH, David OA, Terna AD. Green synthesis and characterization of silver nanoparticles using *Morinda lucida* leaf extract and evaluation of its antioxidant and antimicrobial activity. Chem Pap. 2022; 76(12): 7313–7325.

52 Hossain MR, Biplob AI, Sharif SR, Bhuiya AM, Sayem ASM. Antibacterial activity of green synthesized silver nanoparticles of *Lablab purpureus* flowers extract against human pathogenic bacteria. Trop J Nat Prod Res. 2023; 7(8): 3647–3651.

53 Anuar AHH, Ghafar SAA, Lim V, Pazli NFAM, Hanafiah RM. Critical evaluation of green synthesized silver nanoparticles-kaempferol for antibacterial activity against methicillin-resistant *Staphylococcus aureus*. Int J Nanomedicine. 2024; 19: 1339–1350.