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Biogenic Nanoparticles synthesized from *Murraya koenigii* (l.) Spreng. and their Broad-spectrum Antimicrobial activity

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Abstract

The extensive use of synthetic pesticides for controlling plant diseases has raised concerns regarding environmental safety and the development of resistant microbial populations, prompting the search for sustainable alternatives. In the present study, silver nanoparticles (AgNPs) were synthesized using the aqueous leaf extract of Murraya koenigii (L.) Spreng. through a green synthesis approach and evaluated for their antimicrobial activity against selected phytopathogenic bacteria and fungi. The formation of AgNPs was visually indicated by the transformation of the reaction mixture from pale yellow to dark brown and was confirmed by an absorption peak at approximately 430nm in the UV–Visible spectrum. Fourier Transform Infrared spectroscopy suggested the involvement of naturally occurring phytochemicals in the reduction and stabilization of nanoparticles, while Scanning Electron Microscopy revealed predominantly spherical particles ranging from 20 to 45nm in size. The antimicrobial potential of the synthesized AgNPs was investigated against Xanthomonas oryzae pv. oryzae, Xanthomonas campestris pv. vesicatoria, Xanthomonas campestris pv. campestris, Pseudomonas syringae, Aspergillus flavus and Fusarium verticillioides. The nanoparticles suppressed the growth of all tested pathogens in a concentration-dependent manner, with the highest antibacterial activity recorded against Xanthomonas oryzae pv. oryzae at 100µg mL⁻¹. Minimum inhibitory concentrations ranged from 25 to 75µg mL⁻¹, whereas minimum bactericidal/fungicidal concentrations varied between 50 and 150µg mL⁻¹. The results demonstrate that Murraya koenigii-mediated silver nanoparticles possess broad-spectrum antimicrobial activity and may serve as a promising eco-friendly alternative for managing plant diseases. These findings also reinforce the potential of plant-based nanotechnology as a sustainable approach for developing effective antimicrobial agents for agricultural applications.

Keywords: Green synthesis, Silver nanoparticles, *Murraya koenigii*, Phytopathogens, Antimicrobial activity, Nano-biocontrol.

Introduction

Crop production has always been closely linked to the ability to manage plant diseases effectively. Among the many factors that limit agricultural productivity, bacterial and fungal pathogens remain particularly destructive because they not only reduce yield but also affect the quality and marketability of agricultural produce. The challenge has become even greater with the increasing occurrence of resistant pathogen populations and the growing demand for agricultural practices that are both productive and environmentally responsible[1,2].

For decades, disease management has depended largely on synthetic pesticides and antimicrobial chemicals. While these compounds have undoubtedly contributed to improving crop protection, their prolonged use has also raised concerns that extend beyond disease control. Residual chemicals in agricultural ecosystems, adverse effects on beneficial microorganisms, contamination of soil and water resources, and the gradual loss of effectiveness due to microbial resistance have encouraged researchers to reconsider how plant diseases are managed[3,4].

These concerns have shifted scientific attention towards approaches that combine biological safety with effective antimicrobial performance.

One area that has shown remarkable progress in recent years is the application of nanotechnology to agriculture. Metallic nanoparticles, particularly silver nanoparticles (AgNPs), have demonstrated strong antimicrobial activity against a wide range of microorganisms. Unlike conventional antimicrobial compounds that generally affect a single cellular target, silver nanoparticles interact with microbial cells through multiple pathways. They can alter membrane integrity, interfere with enzyme function, promote oxidative stress and disrupt essential cellular processes, collectively reducing the survival and multiplication of pathogenic microorganisms[5–7]. Such diverse mechanisms make them attractive candidates for developing alternative plant protection strategies.

Equally important is the method by which these nanoparticles are produced. Conventional physical and chemical synthesis often relies on expensive instrumentation, high energy requirements or hazardous reducing agents.

In contrast, green synthesis makes use of naturally occurring biomolecules to convert metal ions into stable nanoparticles under relatively mild conditions. Plant extracts are particularly suitable for this purpose because they contain a complex mixture of phytochemicals capable of acting simultaneously as reducing and stabilizing agents. As a result, plant-mediated synthesis offers a simple, economical and environmentally compatible route for producing biologically active nanoparticles[8,9].

Murraya koenigii (L.) Spreng., commonly known as curry leaf, is widely recognized not only as a culinary herb but also as a medicinal plant with a rich diversity of secondary metabolites. The leaves contain carbazole alkaloids, flavonoids, phenolic compounds, terpenoids and several antioxidant constituents that have been associated with antimicrobial and other beneficial biological activities[10].

These naturally occurring compounds also provide a favourable biochemical environment for the formation and stabilization of silver nanoparticles, making Murraya koenigii an appropriate plant source for green nanoparticle synthesis.

Although plant-mediated silver nanoparticles have been investigated using different medicinal species, comparatively fewer studies[11] have examined the antimicrobial potential of *Murraya koenigii*-derived nanoparticles against both bacterial and fungal phytopathogens of agricultural importance within a single investigation. Recent investigations also demonstrate that *Murraya koenigii* continues to receive increasing attention as a promising biological source for multifunctional nanoparticle synthesis and agricultural applications[12,13].

Information comparing their activity against multiple crop pathogens under standardized experimental conditions remains limited[14,15]. Expanding this knowledge is important for assessing their suitability as sustainable antimicrobial agents for plant disease management.

With this background, the present investigation was designed to synthesize silver nanoparticles using the aqueous leaf extract of *Murraya koenigii* and to evaluate their antimicrobial activity against selected bacterial and fungal phytopathogens. The synthesized nanoparticles were characterized by UV–Visible spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy and Scanning Electron Microscopy (SEM), followed by assessment of their antimicrobial efficacy using agar well diffusion, minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) assays.

The study seeks to provide experimental evidence supporting the use of plant-derived silver nanoparticles as environmentally compatible materials for future crop protection and sustainable disease management[16].

Materials and Methods

Collection and Preparation of Plant Material: Fresh leaves of *Murraya koenigii* (L.) Spreng. were collected from healthy plants and thoroughly cleaned under running tap water to eliminate adhering dust and debris. The leaves were rinsed again with distilled water and dried under shade at room temperature until complete removal of moisture. The dried material was finely powdered using a laboratory grinder. Twenty grams of the powdered leaves were transferred to a conical flask containing 200 mL of distilled water and heated at 60°C for 30 min with occasional stirring to facilitate extraction of water-soluble phytochemicals. After cooling to room temperature, the extract was filtered through Whatman No. 1 filter paper, and the clear filtrate was used immediately for nanoparticle synthesis.

Biosynthesis of Silver Nanoparticles: Silver nanoparticles were synthesized using the aqueous leaf extract following a plant-mediated green synthesis procedure with slight modifications[9]. Briefly, 10mL of the prepared extract was added slowly to 90mL of freshly prepared 1mM aqueous silver nitrate ($AgNO_3$) solution while maintaining continuous magnetic stirring. The reaction mixture was protected from light and allowed to stand for 24h at room temperature to facilitate reduction of silver ions. A gradual colour transition from pale yellow to dark brown served as the initial indication of nanoparticle formation, reflecting the development of surface plasmon resonance. The reaction mixture was subsequently centrifuged at 12,000rpm for 15 min. The resulting pellet was washed three times with sterile distilled water to remove unreacted constituents and finally dried to obtain purified silver nanoparticles for further analyses.

Characterization of Silver Nanoparticles: UV–Visible Spectroscopy: Formation of silver nanoparticles was monitored by analysing the reaction mixture using a UV–Visible spectrophotometer[9,17]. Spectral measurements were recorded between 300 and 700nm with distilled water serving as the reference blank. The appearance of a characteristic absorption peak in the visible region confirmed the successful synthesis of silver nanoparticles[18].

Fourier Transform Infrared (FTIR) Analysis: Fourier Transform Infrared spectroscopy was employed to identify the phytochemical functional groups associated with nanoparticle formation. Dried nanoparticle samples were finely blended with spectroscopic-grade potassium bromide (KBr) and scanned over the range of 4000–400cm^{-1}. The obtained spectra were analysed to determine the biomolecules involved in reducing silver ions and stabilizing the synthesized nanoparticles[19].

Scanning Electron Microscopy (SEM): The external morphology and approximate particle size of the synthesized nanoparticles were examined using Scanning Electron Microscopy. A small aliquot of the nanoparticle suspension was placed on a carbon-coated specimen stub and allowed to dry completely. The sample was then sputter-coated with a thin layer of gold before microscopic examination. High-resolution images were recorded to evaluate particle shape, surface characteristics and size distribution[20].

Test Microorganisms: The antimicrobial efficacy of the synthesized silver nanoparticles was evaluated against representative bacterial and fungal phytopathogens of agricultural importance. The bacterial test organisms included *Xanthomonas oryzae* pv. *oryzae*, *Xanthomonas campestris* pv. *vesicatoria*, *Xanthomonas campestris* pv. *campestris* and *Pseudomonas syringae*. Antifungal activity was assessed against *Aspergillus flavus* and *Fusarium verticillioides*. These microorganisms were selected because of their economic significance in crop production and their ability to cause serious plant diseases under field and storage conditions[21].

Assessment of Antimicrobial Activity: Antimicrobial activity was determined using the agar well diffusion technique following standard microbiological procedures[18]. Nanoparticle suspensions were tested at concentrations of 25, 50, 75 and 100 $\mu g\ mL^{-1}$. After incubation, the diameter of the inhibition zone surrounding each well was measured in millimetres. The minimum inhibitory concentration (MIC) was determined using the broth microdilution method as the lowest nanoparticle concentration capable of preventing visible microbial growth. Minimum bactericidal concentration (MBC) for bacteria and minimum fungicidal concentration (MFC) for fungi were subsequently established by subculturing MIC assay samples onto fresh culture media to identify the lowest concentration that completely eliminated viable microorganisms[22].

Statistical Analysis: Each experimental treatment was carried out in triplicate to ensure reproducibility. The experimental data are presented as mean ± standard deviation (SD). Statistical comparisons among treatments were performed using one-way analysis of variance (ANOVA), and differences were considered statistically significant when the probability value was less than 0.05.

Results and Discussion

Biosynthesis and Characterization of Silver Nanoparticles: The formation of silver nanoparticles was visually evident from the gradual transformation of the reaction mixture from pale yellow to dark brown following the addition of the aqueous leaf extract of *Murraya koenigii* to the silver nitrate solution. No colour change was observed in the control solution lacking plant extract. The appearance of the brown colour is a characteristic indication of silver nanoparticle formation and results from the excitation of surface plasmon resonance (SPR), which occurs when free electrons on the nanoparticle surface interact with incident light[9,17]. This preliminary observation suggested the efficient reduction of silver ions (Ag^{+}) into metallic silver (Ag^{0}) by phytochemicals present in the leaf extract.

UV–Visible Spectral Analysis: The UV–Visible absorption spectrum of the synthesized nanoparticles displayed a distinct absorption maximum between 420 and 450nm (Figure-1), a region typically associated with the surface plasmon resonance of silver nanoparticles. The presence of a single, well-defined absorption peak indicated successful nanoparticle formation and suggested that the particles were relatively uniform in size with limited aggregation. The absence of additional broad peaks further reflected the stability of the colloidal suspension under the experimental conditions. Comparable absorption profiles have been reported for silver nanoparticles synthesized using plant extracts, confirming that the observed spectral characteristics are consistent with biologically synthesized AgNPs[6,9,18].

Fourier Transform Infrared (FTIR) Analysis: FTIR analysis was carried out to identify the functional groups associated with nanoparticle formation and stabilization. The spectrum exhibited a broad absorption band near $3400cm^{-1}$, which corresponds to the stretching vibration of hydroxyl (–OH) groups commonly present in phenolic compounds and alcohols. A prominent band around $1630cm^{-1}$ was attributed to carbonyl (C=O) stretching vibrations, whereas the absorption peak near $1050cm^{-1}$ was assigned to C–O stretching of alcohols and phenolic constituents (Figure-2).

These spectral features indicate that naturally occurring phytochemicals present in Murraya koenigii leaf extract participated in the reduction of silver ions and subsequently remained adsorbed on the nanoparticle surface as stabilizing molecules. Such biomolecules not only facilitate nanoparticle synthesis but also improve colloidal stability by minimizing particle aggregation during and after the reduction process. Plant-mediated synthesis offers a simple, economical and environmentally compatible route for producing biologically active nanoparticles. Recent reviews further emphasize that advances in green nanotechnology have expanded the agricultural applications of silver nanoparticles, including plant disease management, stress tolerance and sustainable crop protection[8,22,23].

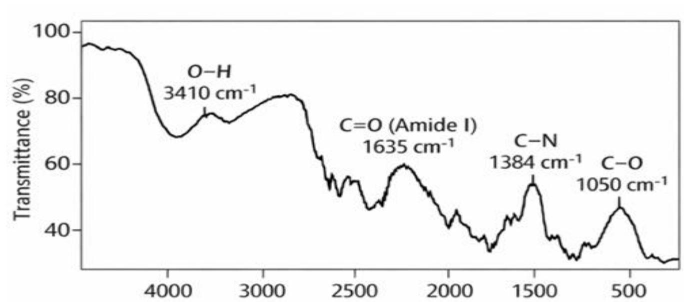

Figure-1: FTIR spectral analysis of plant extract and synthesized plant extract Nano particles exhibiting involvement of different plant biomolecules in the reduction and stabilization process.

Scanning Electron Microscopy (SEM): The surface morphology of the biosynthesized silver nanoparticles was examined using Scanning Electron Microscopy (SEM). The micrographs demonstrated that the majority of the nanoparticles possessed a nearly spherical morphology with particle sizes ranging from approximately 20 to 60nm (Figure-2). Most particles appeared to be well dispersed, although a few localized clusters were observed. Such aggregation is commonly encountered in biologically synthesized nanoparticles and may result from interactions among phytochemical molecules adsorbed on the nanoparticle surface during the capping process[9,20].

The relatively small particle size obtained in the present investigation is advantageous because nanoparticles with a larger surface area generally exhibit greater interaction with microbial cell surfaces, thereby improving their antimicrobial effectiveness[5,9]. The observed morphology and size distribution are in close agreement with earlier reports describing silver nanoparticles synthesized using plant extracts, indicating that the green synthesis protocol adopted in this study produced stable nanostructures suitable for biological applications[6,9].

Collectively, the findings obtained from UV–Visible spectroscopy, FTIR analysis and SEM characterization confirmed the successful biosynthesis of silver nanoparticles using the aqueous leaf extract of *Murraya koenigii*. The spectroscopic and microscopic evidence demonstrated that plant-derived phytochemicals were actively involved in both the reduction of silver ions and the stabilization of the resulting nanoparticles. These observations further support the effectiveness of plant-mediated synthesis as a simple, environmentally compatible and sustainable approach for producing biologically active silver nanoparticles[6,9].

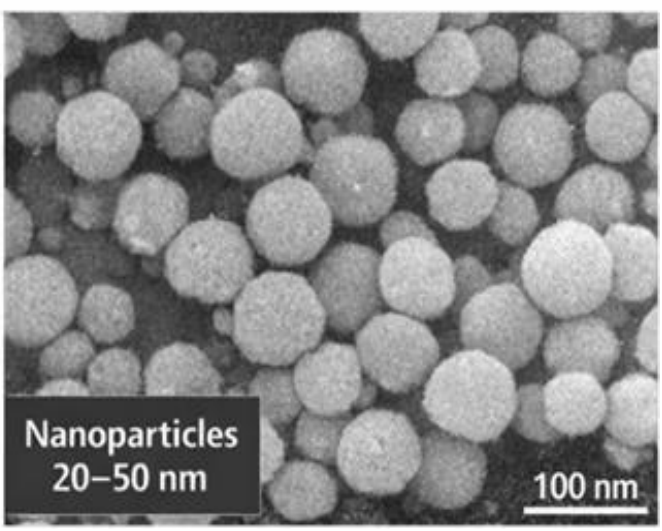

Figure-2: SEM micrograph showing predominantly spherical silver nanoparticles with particle sizes ranging from approximately 20–50 nm. The scale bar represents 100 nm.

Antibacterial Activity of Synthesized Silver Nanoparticles: The antibacterial efficacy of the biosynthesized silver nanoparticles was evaluated against four economically important phytopathogenic bacteria, and the results are presented in Table-1. The nanoparticles inhibited the growth of all tested bacterial species, with the magnitude of inhibition increasing progressively as the nanoparticle concentration increased from 25 to 100µg mL^{-1}. This concentration-dependent response indicates that higher nanoparticle levels enhanced their interaction with bacterial cells, thereby improving antimicrobial effectiveness.

Among the bacterial pathogens evaluated, *Xanthomonas oryzae* pv. *oryzae* exhibited the greatest susceptibility to the synthesized AgNPs, producing the largest inhibition zone (24.3 ± 0.8mm) at 100µg mL^{-1}. *Xanthomonas campestris*pv. *vesicatoria* and *Xanthomonas campestris*pv. *campestris* also responded strongly, recording inhibition zones of 22.4 ± 0.7 mm and 21.1 ± 0.6mm, respectively. Although *Pseudomonas syringae* was comparatively less sensitive, a distinct inhibitory effect was still evident, with a maximum inhibition zone of 19.8 ± 0.7 mm at the highest concentration tested. These observations demonstrate that the synthesized nanoparticles possess broad-spectrum antibacterial activity against diverse phytopathogenic bacteria.

Further evidence of their antimicrobial potential was obtained from the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays. The MIC values ranged between 25 and 75µg mL^{-1}, whereas complete bactericidal activity was achieved at concentrations ranging from 50 to 150µg mL^{-1}. The comparatively lower MIC recorded for *Xanthomonas oryzae* pv. *oryzae* indicates that this pathogen required a smaller quantity of nanoparticles to suppress visible bacterial growth, reflecting its greater susceptibility to the synthesized AgNPs. Comparable findings have been reported for plant-mediated silver nanoparticles against Gram-negative phytopathogens, where effective inhibition was achieved at relatively low concentrations[5,6].

The antibacterial activity observed in the present investigation can be attributed to the multifaceted mode of action of silver nanoparticles. Owing to their nanoscale dimensions and large reactive surface area, AgNPs readily attach to the bacterial cell envelope, alter membrane permeability and promote the generation of reactive oxygen species. These events disrupt essential metabolic processes, interfere with protein and nucleic acid function, and ultimately result in irreversible cellular damage and bacterial death[5,9]. The pronounced activity observed against *Xanthomonas* species further suggests that the synthesized nanoparticles may have considerable potential for managing bacterial diseases affecting economically important crops.

Table-1: Antimicrobial activity of green synthesized silver nanoparticles (AgNPs) derived from *Murraya koenigii* leaf extract against selected phytopathogenic bacteria and fungi showing zone of inhibition (mm), minimum inhibitory concentration (MIC), and minimum bactericidal/fungicidal concentration (MBC/MFC) at different concentrations (25–100 µg/mL).

Test Organism	Abbreviation	Concentration (µg/mL)	Zone of Inhibition (mm) Mean ± SD	MIC (µg/mL)	MBC/MFC (µg/mL)
Xanthomonas campestris pv. *Vesicatoria*	X. c. pv. v.	25	9.2 ± 0.4	50	100
		50	14.8 ± 0.6		
		75	18.6 ± 0.5		
		100	22.4 ± 0.7		
Xanthomonas campestris pv. *Campestris*	X. c. pv. c.	25	8.6 ± 0.5	50	100
		50	13.9 ± 0.7		
		75	17.5 ± 0.4		
		100	21.1 ± 0.6		
Xanthomonas oryzae pv. *oryzae*	X. o. pv. o.	25	10.4 ± 0.6	25	50
		50	16.7 ± 0.5		
		75	20.9 ± 0.6		
		100	24.3 ± 0.8		
Pseudomonas syringae	—	25	7.9 ± 0.3	75	150
		50	12.3 ± 0.5		
		75	16.4 ± 0.6		
		100	19.8 ± 0.7		
Aspergillus flavus	—	25	6.8 ± 0.4	100	200
		50	10.5 ± 0.6		
		75	14.9 ± 0.5		
		100	18.7 ± 0.6		
Fusarium verticillioides	—	25	7.2 ± 0.3	75	150
		50	11.8 ± 0.4		
		75	15.6 ± 0.6		
		100	19.4 ± 0.7		

Conclusion

Overall, the findings demonstrate that *Murraya koenigii*-mediated silver nanoparticles possess strong antibacterial activity against the phytopathogens investigated. Their effectiveness at relatively low concentrations, together with the environmentally benign nature of the synthesis process, highlights their potential as a sustainable alternative to conventional chemical bactericides for future plant disease management strategies[6-9].

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