Lakshmi et al | Article 10261
J Pure Appl Microbiol. 2025;19(2):1511-1527. doi: 10.22207/JPAM.19.2.56
Received: 24 January 2025 | Accepted: 23 April 2025
Published Online: 04 June 2025

P-ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

Phytobiologic Mediated Synthesis of Bactericidal Nanoparticles and their Multifaceted Applications with Metabolomics Insights Via GC-MS

S. Lakshmi[1], Manaveya Honawad[1], L.R. Monisha[1], S. Roopanjali[2], H. Shayista[1], Vaibhavi J Godkhindi[1], M.N. Nagendra Prasad[1], S. Niranjan Raj[3], K. Manju[3], P. Akshatha[2], Kshithij G Raj[2], Ravikumara[3] and Syed Baker[3]*

[1]Department of Biotechnology, JSS Science and Technology University, Mysore, Karnataka, India.
[2]Department of Civil Engineering, JSS Science and Technology University, Mysore, Karnataka, India.
[3]Department of Studies in Microbiology, Karnataka State Open University, Mysore, Karnataka, India.

Abstract

The study mediates silver nanoparticles (AgNPs) synthesis from *Euphorbia geniculata* and is evaluated for its multifunctional applications in healthcare, agriculture, and environmental sustainability. The AgNPs showed bactericidal activity against an array of human pathogens, including *Escherichia coli*, *Salmonella typhi*, and *Bacillus cereus,* with a 1.3 cm zone of inhibition and a 1.1 cm zone of inhibition against *Staphylococcus aureus*. The antioxidant potential of AgNPs were authenticated with the DPPH assay, which demonstrated scavenging activity of 43.22% at 75 mg. Plant growth promotion of AgNPs was evaluated, which showed an increase in the root and shoot length. The AgNPs also displayed dye degradation efficacy with a time interval of 24 hours. The AgNPs were biophysically characterized via spectroscopic analysis, which depicted a maximum peak at 418 nm. FTIR analysis revealed the functional groups at different frequencies, with major groups identified as hydroxyl and carboxyl groups. The size distribution pattern of AgNPs was studied with DLS analysis, showing the size of the particles as 201 nm. The morphological characterisation using TEM showed polydispersity from 10 nm to 100 nm. Additionally, XRD results proved the crystalline structure of synthesised AgNPs, showing the 2θ peaks at 38°, 44°, 64°, and 77°. The Phyto metabolomic studies of *Euphorbia geniculata* showed 40 different active phytocomponents in the methanolic extract. Some of the major metabolites include derivatives of 1-butanol, oleic acid, and n-hexadecanoic acid through GC-MS analysis. Overall, the study demonstrates the multifunctional properties of nanoparticles with profound activities.

Keywords: *Euphorbia geniculata*, Silver Nanoparticles, Dye Degradation, Plant Growth Promotion

***Correspondence:** syedbaker3@gmail.com

Citation: Lakshmi S, Honawad M, Monisha LR, et al. Phytobiologic Mediated Synthesis of Bactericidal Nanoparticles and their Multifaceted Applications with Metabolomics Insights Via GC-MS. *J Pure Appl Microbiol.* 2025;19(2):1511-1527. doi: 10.22207/JPAM.19.2.56

INTRODUCTION

Recent advances in nanoscience have revolutionized multiple scientific disciplines by enabling the production of multifunctional nanoparticles with enhanced physicochemical and biological potential.[1] Among various nanomaterials, AgNPs have gained importance due to their unique optical, catalytic, and biological characteristics.[2] AgNPs exhibit a broad spectrum of bioactivities, including potent antibacterial, antifungal, antiviral, antioxidant, and anticancer properties. Thus, leading to applications in healthcare, agriculture, and environmental remediation.[3]

A critical aspect in realising the potential of AgNPs lies in the synthesis protocol, which not only dictates the particle's morphology and bioactivity but also determines its environmental footprint. Green synthesis approaches using biological systems have become evident as sustainable substitutes to conventional chemical and physical procedures in this context.[4] Plant-mediated synthesis, in particular, offers numerous advantages such as simplicity, cost-effectiveness, scalability, and environmental benignity.[5] Moreover, plants serve as a dual source of both reducing and stabilising agents through their diverse phytochemical constituents, eliminating the need for toxic reagents.[6]

Green-synthesised AgNPs are emerging as pivotal components in next-generation biomedical technologies due to their superior biocompatibility, surface functionalization potential, and controlled physicochemical attributes.[7] A study demonstrated the synthesis of AgNPs by mixing aqueous extracts of *Paullinia cupana* Kunth and $AgNO_3$ (1 mM) in a 1:9 ratio and heating at 70 °C for 180 mins. These nanoparticles were effective against several bacterial and fungal strains.[8] Pirtarighat *et al.*[9] synthesized AgNPs by treating *Salvia spinosa* extract with $AgNO_3$ (1 mM) in the ratio 1:9 and stirring it continuously for 6 hours. The inhibitory function of these nanoparticles was effective against Gram- and Gram+ bacteria. In another study, antifungal AgNPs were produced by dropwise adding *Avena fatua* extract to $AgNO_3$ stock solution and stirring on a magnetic plate for 3 hours.[10] In similar terms, Asif *et al.*[11] synthesized AgNPs by dropwise adding *Moringa oleifera* leaves extract in 100 ml $AgNO_3$ solution in the temperature range 60 °C-80 °C for an hour. 3 mM $AgNO_3$ treated with *Azadirachta indica* extract in the ratio 1:5, followed by incubation in the dark for 24 hours, synthesized AgNPs which were strong inhibitors of *P. aeruginosa*.[12] Phanse *et al.*[13] reported confirmation of AgNPs by mixing $AgNO_3$ and *Tinospora cordifolia* leaf extract in the ratio 8:2 followed by heating at 75 °C for 25 mins.

These nanoparticles have been successfully integrated into multifunctional wound dressings that exhibit enhanced antimicrobial efficacy, accelerated epithelialization, and prevent wound infection, thereby facilitating rapid tissue regeneration.[14-16] Furthermore, their surface-modifiable nature enables conjugation with therapeutic agents, making them efficient carriers in drug delivery systems, particularly for site-specific cancer therapy and antimicrobial interventions.[17-20] In tissue engineering, AgNPs are increasingly incorporated into bioactive scaffolds to promote cell adhesion, proliferation, and extracellular matrix remodelling, while concurrently protecting implant-associated infections.[21] Moreover, their unique surface plasmon resonance properties have facilitated their application in high-sensitivity biosensors for the early detection of biomarkers, pathogens, and environmental toxins.[22,23]

Additionally, the intrinsic antioxidant potential of AgNPs offers therapeutic benefits in reducing oxidative stress, a key contributor to chronic and degenerative diseases. Beyond medical applications, AgNPs synthesized via green routes have shown promise in agricultural systems, where they function as intelligent nano-formulations for sustainable crop management.[24-26] These include nano-enabled pesticides with controlled release profiles and enhanced target specificity. Recent studies highlight nano-fertilizers that optimize nutrient bioavailability and uptake efficiency, and nanoscale diagnostic platforms for real-time detection of plant pathogens through colorimetric, electrochemical, or fluorescence-based sensing modalities. Such precision agriculture interventions not only enhance crop yield and quality but also reduce agrochemical runoff and environmental toxicity, aligning with the principles of circular bioeconomy and sustainable farming practices.[27,28] Moreover, environmental applications of AgNPs are increasingly being

recognised, particularly in wastewater treatment, where their catalytic properties facilitate the decomposition of hazardous synthetic dyes like methylene blue (MB) and crystal violet (CV) into less toxic byproducts.[29,30] These technologies represent a fast-advancing field of applied research with significant societal impact.

Among various plant species explored for nanoparticle biosynthesis, the genus *Euphorbia* stands out due to its rich reservoir of bioactive metabolites, including flavonoids, terpenoids, phenolics, and alkaloids.[31] These metabolites reduce metal ions and act as stabilizing agents for the nanoparticles, thereby enhancing their physicochemical stability and biological efficacy.[32] While extensive studies have focused on several *Euphorbia* species, the role of *Euphorbia geniculata* in nanoparticle biosynthesis remains underexplored. However, preliminary phytochemical profiling of *Euphorbia geniculata* reveals a repertoire of secondary metabolites comparable to other Euphorbia species, suggesting its potential as an effective biogenic source.[33] The integration of *Euphorbia geniculata*-mediated AgNPs could open new avenues in combating multidrug-resistant (MDR) pathogens, an escalating global health concern.[34-36]

Given these multidimensional applications, the current research seeks to achieve the synthesis of multifunctional AgNPs using *Euphorbia geniculata* as a green biogenic source. This approach not only harnesses the phytochemical richness of the plant but also lines up with the principles of sustainable nanotechnology, offering a novel strategy for addressing contemporary challenges in health, agriculture, and environmental management.

MATERIALS AND METHODS

The healthy plant materials of *Euphorbia geniculata* were obtained from an abundantly growing area of Mysuru and transported to the laboratory for further processing. The chemicals used in this study were of analytical grade, including $AgNO_3$ (Sigma-Aldrich), DPPH (2,2-Diphenyl-1-Picrylhydrazyl) (SRL), Crystal violet (Sigma Aldrich), Methylene Blue (Sigma Aldrich), Methanol (CH_3OH) (Merck), and Ascorbic acid ($C_6H_8O_6$) (Sigma-Aldrich). Additionally, seeds of *Vigna radiata* and *Cicer arietinum* were obtained from the local market.

Methods

Plant processing

The healthy plant materials were repeatedly washed to remove any residual impurities. Following this, the plant materials were processed to obtain leaf extract by boiling 20 g in 100 ml of sterile water at 80 °C until the colour changed, and the filtrate was separated.[37]

AgNPs and their characterisation

The AgNPs were synthesised by treating $AgNO_3$ with plant extract in a 9:1 ratio with slight modifications as reported in Lima *et al.*[8] The mixture was processed at an elevated temperature above 60 °C. A transition from yellow to reddish-brown was observed, which was then subjected to Ultracentrifugation. The AgNPs obtained in the pellet were analysed through UV-visible spectroscopy for confirmation. Fourier Transform Infrared Spectroscopy (FTIR) analysis identified functional groups between 400 to 4000 cm^{-1}. X-ray Diffraction (XRD) was conducted to check the crystal characteristics. This experiment used an X-ray diffractometer to check the 2θ angles from 20 degrees to 100 degrees. The morphological analysis and size distribution of AgNPs were studied using TEM (Transmission Electron Microscopy)[38] and DLS (Dynamic Light Scattering), respectively.

Antibacterial Activity of *Euphorbia geniculata*-Mediated AgNPs

The bactericidal activity was assessed by evaluating the AgNPs in the agar well diffusion method. Uniform swabbing of test pathogens followed by punching of agar to create wells, which were seeded with AgNPs at a 50 mg/mL concentration. Incubation was carried out at 37 °C for 24 hours. A clear zone of inhibition across the well showed the bactericidal activity, which was compared with standard antibiotics.[39]

Antioxidant Assay of *Euphorbia geniculata*-Mediated AgNPs

The antioxidant potential of *Euphorbia geniculata-mediated* AgNPs were assessed using the DPPH assay. Here, ascorbic acid was set as

a standard antioxidant. AgNPs solutions ranging from 25, 50, and 75 µg/mL were prepared by dispersing the *Euphorbia geniculata*-mediated AgNPs in 1 mL of methanol. The activity was measured by dissolving DPPH solution at a 1 mg / ml concentration, and the activity was measured at 517 nm.[40] The activity was measured with the following formula

$$\% \text{ inhibition} = A\text{-}B / A \times 100$$

A: Control absorbance
B: Sample

Plant Growth Promotion Assay and Chlorophyll Content Measurement of *Euphorbia geniculata*-Mediated AgNPs

The potential of *Euphorbia geniculata-mediated* AgNPs to promote plant growth was assessed using *Vigna radiata* and *Cicer arietinum* seeds. The seeds were surface sterilized and soaked at a concentration of 10 mg/ml of nanoparticles overnight.[38-41] Sterilised soil (approximately 50 g) was distributed into sterile containers, and both AgNPs-treated and control seeds were sown separately. The containers were kept in a well-lit environment to ensure favourable growth conditions for 7 days. Upon completion of the growth period, the plants were gently removed to prevent damage to their root and shoot structures. Measurements of root and shoot lengths were then recorded to evaluate the influence of AgNPs treatment on plant development.[42]

Chlorophyll content was determined by mechanically grinding the leaf material of the plants using chloroform. The content was measured at 645 and 663 nm.[43] This analysis provided insights into the physiological effects of the *Euphorbia geniculata*-mediated AgNPs on chlorophyll production and overall plant health.

• Chlorophyll a (Chl A)

$$\text{Chl A} = 12.25 \times A_{663} - 2.79 \times A_{645}$$

• Chlorophyll b (Chl B)

$$\text{Chl B} = 21.50 \times A_{645} - 5.10 \times A_{663}$$

• Total Chlorophyll (Total Chl)

$$\text{Total Chl} = 20.2 \times A_{645} + 8.02 \times A_{663}$$

Dye Degradation Assay of *Euphorbia geniculata*-Mediated AgNPs

The photodegradation potential of *Euphorbia geniculata*-mediated AgNPs was evaluated using selected dyes, crystal violet (CV),

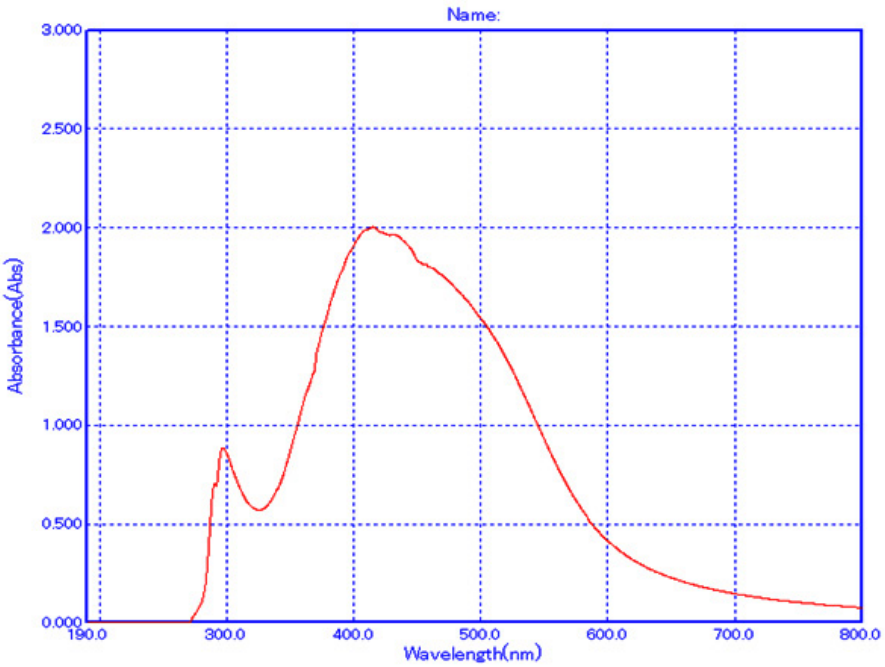


Figure 1. Spectroscopic analysis of *Euphorbia geniculata*-Mediated AgNP's

and methylene blue (MB). The stock solutions were prepared with 1 mg/ml, and 10 µL of the *Euphorbia geniculata*-mediated AgNPs were mixed with the dyes, and incubated under sunlight for 24 hrs. The degradation potential was measured at 663 nm for MB and 590 nm for CV. After 24 hours, the final absorbance values were recorded to evaluate the extent of degradation, with a decrease indicating successful nanoparticle-mediated degradation.[38,44]

Soxhlet Extraction and GC-MS Analysis of *Euphorbia geniculata* Crude Extract

The Soxhlet extraction of *Euphorbia geniculata* was carried out as per the procedure outlined by Hatami.[45] A 20 g sample with 250 ml of methanol was subjected to extraction using a standard Soxhlet apparatus. This process was conducted at 65 °C. The obtained crude extract was studied via GC-MS, which used a fused silica column, and the carrier gas was helium. The reaction was performed at 250 °C.[46] The resulting spectral data were compared with the NIST 2017 GC-MS library for compound identification.[47]

RESULTS

The results of *Euphorbia geniculata*-mediated AgNPs were visually verified by the

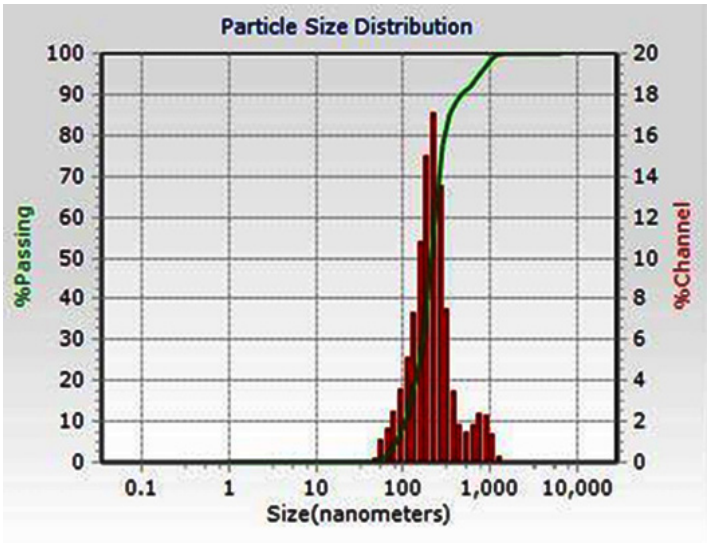


Figure 2. Dynamic light scattering graph of *Euphorbia geniculata*-Mediated AgNP's

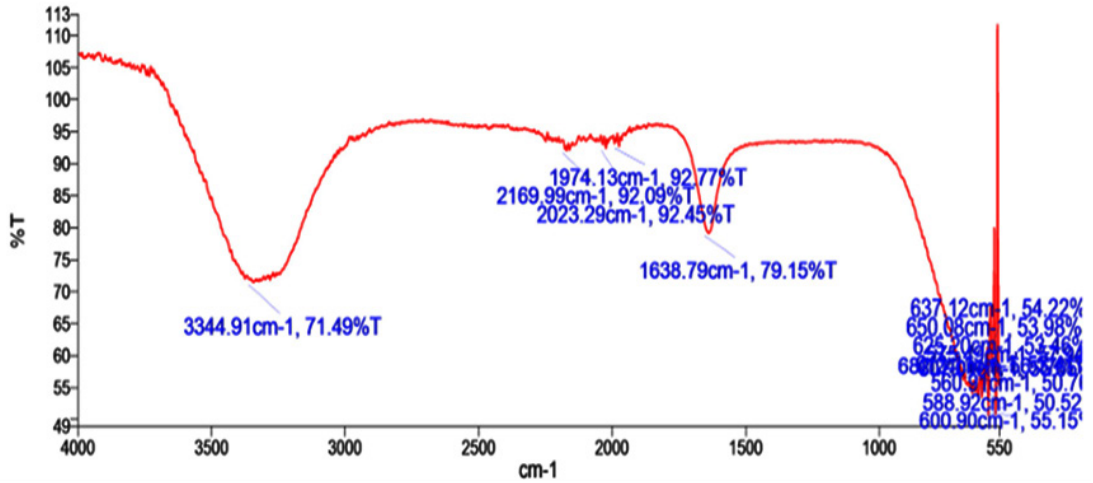


Figure 3. FTIR graph showing peaks of functional groups in *Euphorbia geniculata*-Mediated AgNP's

change in colour to reddish-brown, which might be due to the excitation of electrons. This was confirmed with UV-visible spectroscopy, with a distinct peak at 418 nm, a standard absorption wavelength for AgNPs, as shown in Figure 1.

Characterization of *Euphorbia geniculata*-mediated AgNPs

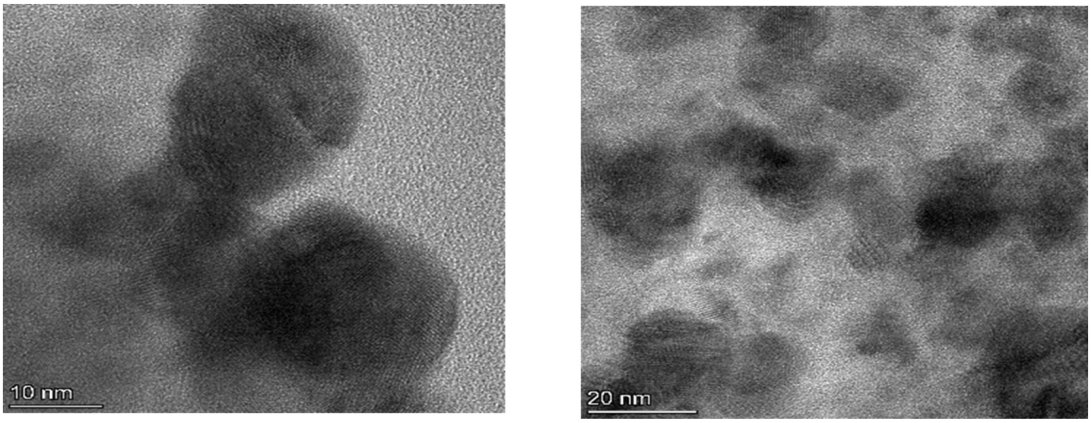
The DLS analysis of *Euphorbia geniculata*-mediated AgNPs revealed a polydisperse size

Table 1. DPPH Activity of *Euphorbia geniculata*-Mediated AgNP's

Time	Sample (mg/mL)	OD (517 nm)	Percentage
1 hour	25	1.812	33.28%
	50	1.765	35.01%
	75	1.542	43.22%

Table 2. Chlorophyll measurement of *Cicer arietinum*

Chlorophyll	Control (mg/mL)	Sample (mg/mL)
Chlorophyll A	6.411	10.375
Chlorophyll B	2.0289	14.4853
Total Chlorophyll	9.28572	28.3684



(a) (b)

Figure 4. (a) TEM analysis of the nanoparticles measurement with 10 nm scale range; (b) TEM analysis of the nanoparticles measurement with 20 nm scale range

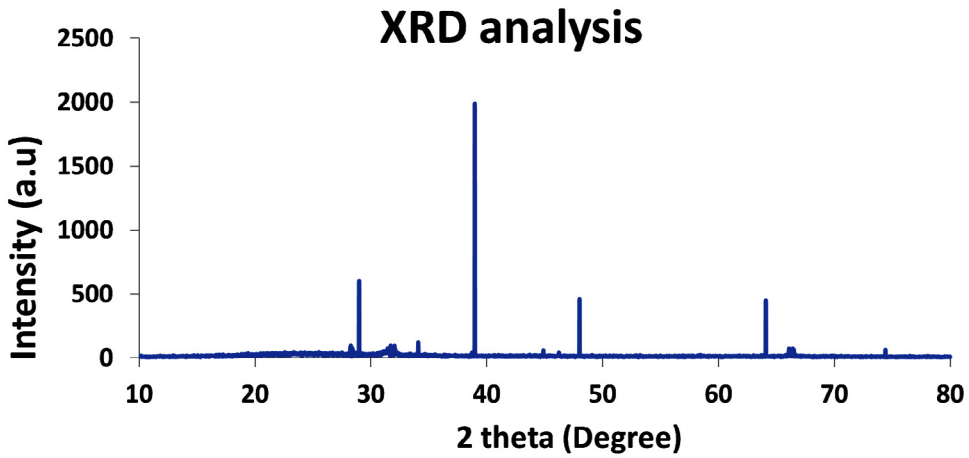


Figure 5. XRD analysis of *Euphorbia geniculata*-Mediated AgNP's

distribution, which displayed an average size of 201 nm with a zeta potential of 0.8 mV, as shown in Figure 2. These functional groups were confirmed by specific absorption peaks within the 600 cm^{-1} to 4000 cm^{-1} as shown in Figure 3. The TEM analysis further checked the morphological characteristics of polydisperse AgNPs with various sizes from 10 to 100 nm, as shown in Figure 4. The FTIR analysis identified several functional groups, including hydroxyl (-OH), alkenes (C=C), carbonyl (C=O), alkynes (C≡C), and halogenated groups (-C-Br, -C-Cl), which are likely involved as capping agents in the nanoparticle synthesis. The XRD analysis confirmed the crystalline nature of the synthesized AgNPs, with distinct diffraction peaks corresponding to the structure of metallic silver. The observed peaks at 38° (111), 44° (200), 64° (220), and 77° (311) are in strong agreement with the standard reference pattern for silver (JCPDS file No. 04-0783), validating the presence of elemental silver in the sample as shown in Figure. 5

Table 3. Chlorophyll measurement of *Vigna radiata*

Chlorophyll	Control (mg/mL)	Sample (mg/mL)
Chlorophyll A	3.39113	7.21257
Chlorophyll B	7.8015	10.0545
Total Chlorophyll	12.1982	18.8631

Antimicrobial activity of *Euphorbia geniculata*-mediated AgNPs

The agar well-diffusion assay revealed significant antimicrobial activity of *Euphorbia*

Table 4. Dye degradation analysis of *Euphorbia geniculata*-Mediated AgNP's

Dye	Duration	Control Absorbance	Sample Absorbance	Degradation Percentage (%)
Crystal violet	2 hours	1.339	1.147	14.4
	24 hours	1.292	0.924	28.2
Methylene blue	1 hour	1.718	1.564	8.96
	2 hours	1.675	1.428	14.76
	24 hours	1.447	1.109	23.4

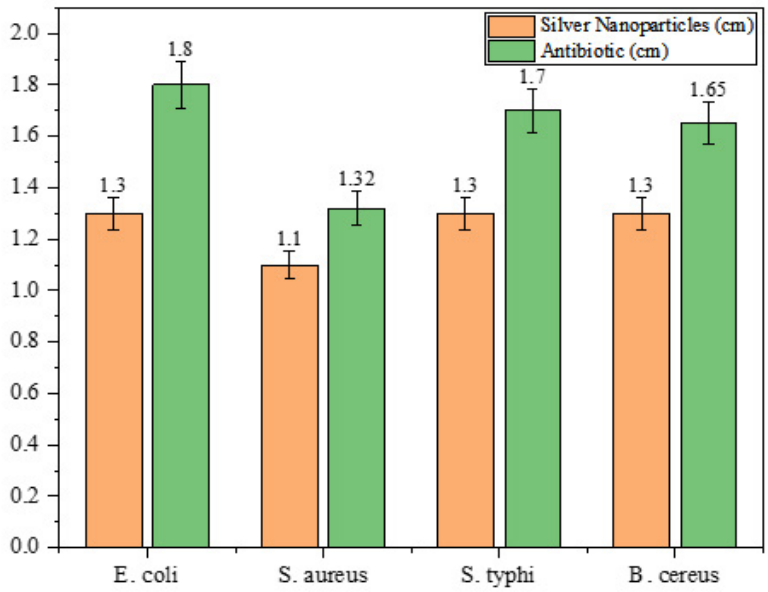


Figure 6. Antimicrobial activity of *Euphorbia geniculata*-Mediated AgNP's and amoxicillin against pathogens via well diffusion

geniculata-mediated AgNPs against the selected human pathogens. Amoxicillin served as the control in the study. The zone of inhibition, measured in centimetres, indicated comparable activity of AgNPs across all pathogens, with the highest inhibition observed against *Escherichia coli*, *Salmonella typhi*, and *Bacillus cereus* at 1.3 cm each. *Staphylococcus aureus* exhibited a slightly lower inhibition zone of 1.1 cm. The comparative results, presented in Figure 6, highlight the antimicrobial potential of *Euphorbia geniculata*-mediated AgNPs, with zones of inhibition observed in Figure 7.

Antioxidant Activity of *Euphorbia geniculata*-Mediated AgNPs

The DPPH assay demonstrated the potent antioxidant activity of *Euphorbia geniculata*-mediated AgNPs, which showed inhibition percentages of 33.28%, 35.01%, and 43.22%, at 25, 50, and 75 µg/mL, respectively. The reduction in free radicals, as evidenced by the decreasing optical density (OD) values at 517 nm, confirms the significant antioxidant potential of the AgNPs. These findings, detailed in Table 1, highlight the efficacy of the nanoparticles in neutralizing free radicals under the tested conditions.

Plant growth promotion

The application of *Euphorbia geniculata*-mediated AgNPs significantly enhanced the growth of *Cicer arietinum* and *Vigna radiata* seeds compared to untreated seeds shown in Figure 8 and Figure 9. In *Cicer arietinum*, nanoparticle-treated seeds showed an increase in root and shoot length of 24 and 22 cm, respectively. In *Vigna*

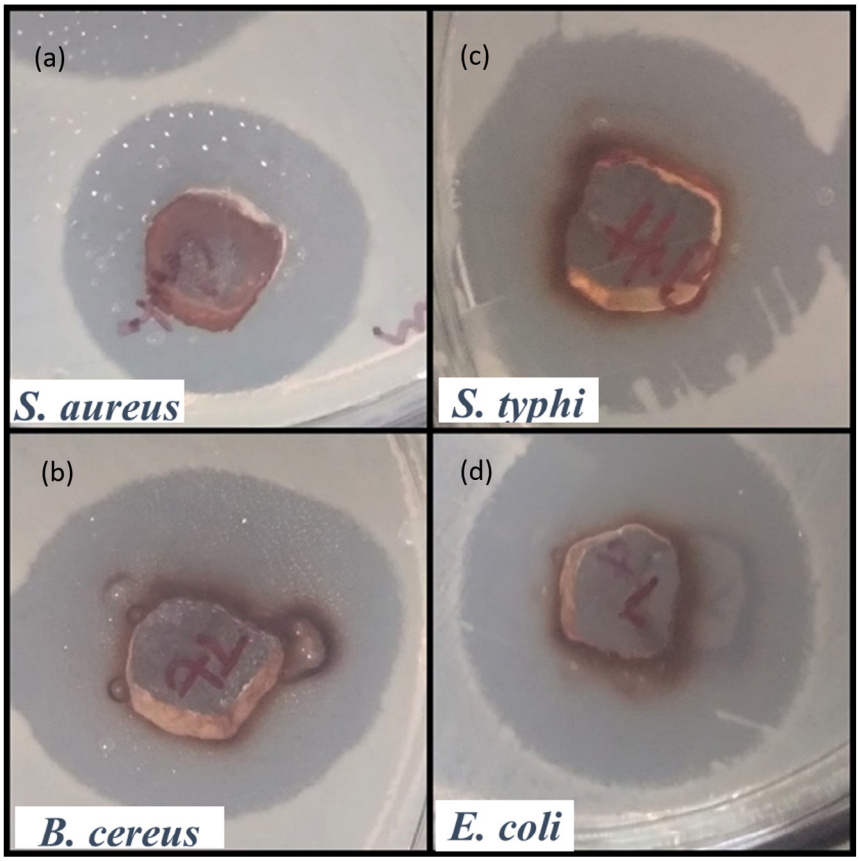


Figure 7. The inhibition zones in well diffusion assay by *Euphorbia geniculata*-Mediated AgNP's. (a) Activity against *S. aureus,* (b) Activity against *B. cereus,* (c) Activity against *S. typhi,* (d) Activity against *E. coli*

radiata, nanoparticle-treated seeds displayed root lengths of 22 cm and shoot heights of 23 cm. Both seed growths were compared with the control group of seeds.

Chlorophyll measurement

The nanoparticle-treated *Cicer arietinum* plants exhibited a significant increase in chlorophyll A, with 10.375 mg/mL compared to the control. Similarly, chlorophyll B levels were higher in the

Table 5. Identified phytochemicals in crude methanol leaf extract of *Euphorbia geniculata*

	Name	Formula	Activity	Molecular Weight
1.	1-Butanol, 3-methyl-, formate	$C_6H_{12}O_2$	Antimicrobial Aromatic compound	116.0837297
2.	2-Propenoic acid, 2 pentyl ester	$C_8H_{14}O_2$	NA	142.09938
3.	1-Butanol, 3-methyl-, acetate	$C_7H_{14}O_2$	Flavour	130.09938
4.	CH3C(O)CH2CH2OH	$C_4H_8O_2$	Flavour and Fragrance	88.0524297
5.	1-Butanethiol, 3-methyl	$C_5H_{12}S$	Odor	104.0659714
6.	2-Butanone, 4-(acetyloxy)	$C_6H_{10}O_3$	Fragrance and Flavour	130.062994
7.	Acrylic acid isoamyl ester	$C_8H_{14}O_2$	NA	142.09938
8.	Methyl tridecyl ether	$C_{14}H_{30}O$	NA	214.229666
9.	1-Dodecanol, 2-octyl-, acetate	$C_{22}H_{44}O_2$	Fragrance	340.334131
10.	2-Isopropyl-4-methylhex-2-enal	$C_{10}H_{18}O$	Fragrance and Flavour	154.135765
11.	Cyclopentane, decyl	$C_{15}H_{30}$	NA	210.234751
12.	Cyclododecanone	$C_{12}H_{22}O$	Fragrance and Flavour	182.167066
13.	2-Octyldecyl acetate	$C_{20}H_{40}O_2$	NA	312.30283
14.	Cyclohexanone, 4-ethyl-3,4-dimethyl	$C_{10}H_{18}O$	NA	154.135765
15.	Oleic Acid	$C_{18}H_{34}O_2$	Antibacterial Antioxidant	282.25588
16.	Pentadecanoic acid	$C_{15}H_{30}O_2$	Anticancer	242.22458
17.	12-Hydroxydodecanoic acid	$C_{12}H_{24}O_3$	Antimicrobial	216.1725445
18.	Z-8-Methyl-9-tetradecenoic acid	$C_{15}H_{28}O_2$	Antifungal	240.20893
19.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Antibacterial Antifungal	256.24023
20.	Undecanoic acid	$C_{11}H_{22}O_2$	Antifungal	186.16198
21.	Cyclopentaneundecanoic acid	$C_{16}H_{30}O_2$	Antioxidant	254.22458
22.	Z-10-Tetradecen-1-ol acetate	$C_{16}H_{30}O_2$	Fragrance and Flavour	254.22458
23.	Methyl Z-11-tetradecenoate	$C_{15}H_{28}O_2$	Antibiotic resistance	240.20893
24.	Methyl9-heptadecenoate or 9-17:1	$C_{18}H_{34}O_2$	NA	282.25588
25.	Methacrylic acid, nonadecyl ester	$C_{23}H_{44}O_2$	NA	352.334131
26.	9-Octadecenamide, (Z)	$C_{18}H_{35}NO$	Anticancer	281.271864
27.	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	NA	296.27153
28.	1-Amininonadecane, N-trifluoroacetyl	$C_{21}H_{40}F_3NO$	Corrosion inhibition	379.3062
29.	Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester	$C_{18}H_{34}O_2$	NA	282.25588
30.	Methyl myristoleate	$C_{15}H_{28}O_2$	NA	240.20893

treated sample by 14.4853 mg/mL than in the control, which showed 2.0289 mg/mL. The total chlorophyll content was also enhanced in the treated plants, with a value of 28.3684 mg/mL compared to the control's 9.28572 mg/mL. This indicates that nanoparticles significantly promote chlorophyll production in *Cicer arietinum* as detailed in Table 2 and Figure 10.

In *Vigna radiata*, the nanoparticle-treated plants showed enhanced chlorophyll A content with 7.21257 mg/mL compared to the control. In similar terms, Chlorophyll B levels were also increased in the treated sample with 10.0545 mg/mL compared to the control, which showed 7.8015 mg/mL. The total chlorophyll content was higher in the treated plants, which showed 18.8631 mg/

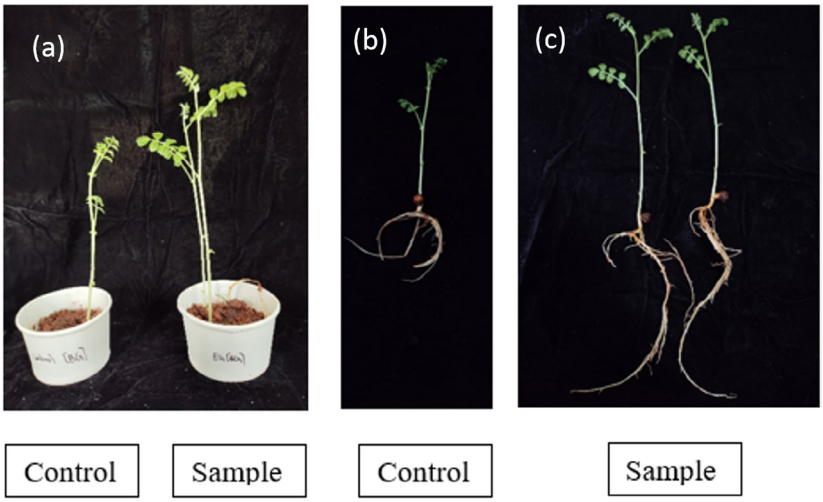


Figure 8. Growth promotion of plant *Cicer arietinum* by *Euphorbia geniculata*-Mediated AgNP's wherein, 8a shows the shoot length measurement. 8b & 8c shows the root length

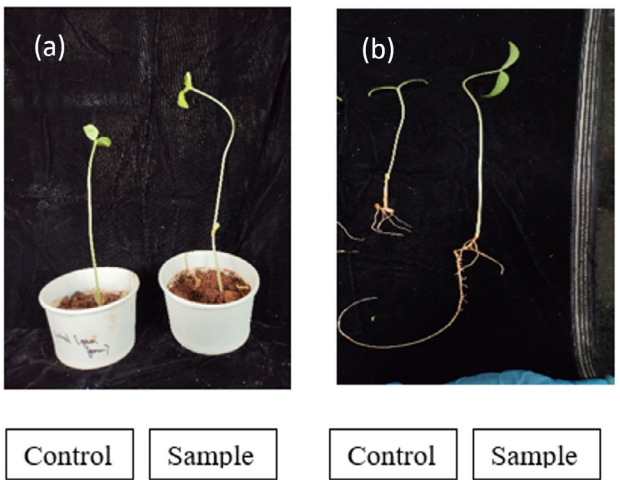


Figure 9. Growth promotion of plant *Vigna radiata* by *Euphorbia geniculata*-Mediated AgNP's wherein, 9a shows the shoot length measurement. 9b shows the root length

mL, than in the control, displaying 12.1982 mg/mL, highlighting the positive effect of nanoparticles on chlorophyll accumulation in *Vigna radiata* as detailed in Table 3 and Figure 11.

Dye degradation assay of *Euphorbia geniculata*-mediated AgNPs

AgNPs exhibited significant dye degradation for both crystal violet and methylene blue, though with varying efficiencies. For crystal violet, the degradation was more pronounced, showing a 14.4% reduction in absorbance after 2 hours and 28.2% after 24 hours. In contrast, the control sample exhibited absorbance values of 1.339 (2 hours) and 1.292 (24 hours), with lesser degradation observed.

Methylene blue degradation was comparatively lower, with 8.96% after 1 hour, 14.76% after 2 hours, and 23.4% after 24 hours. The control sample for methylene blue showed

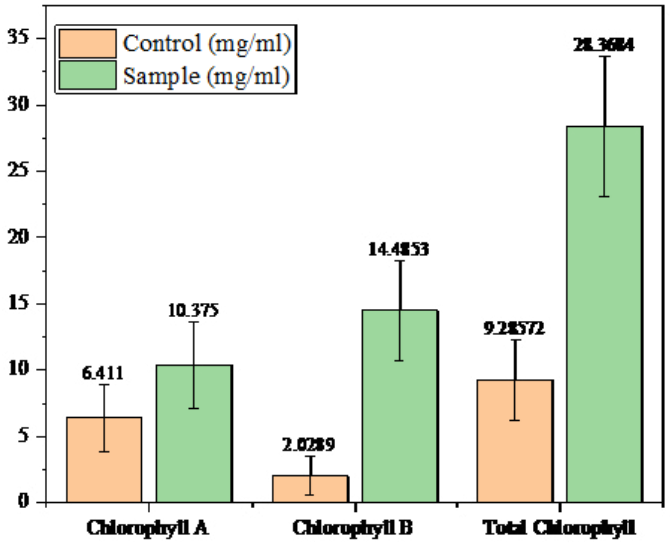


Figure 10. Chlorophyll Content of *Cicer arietinum*

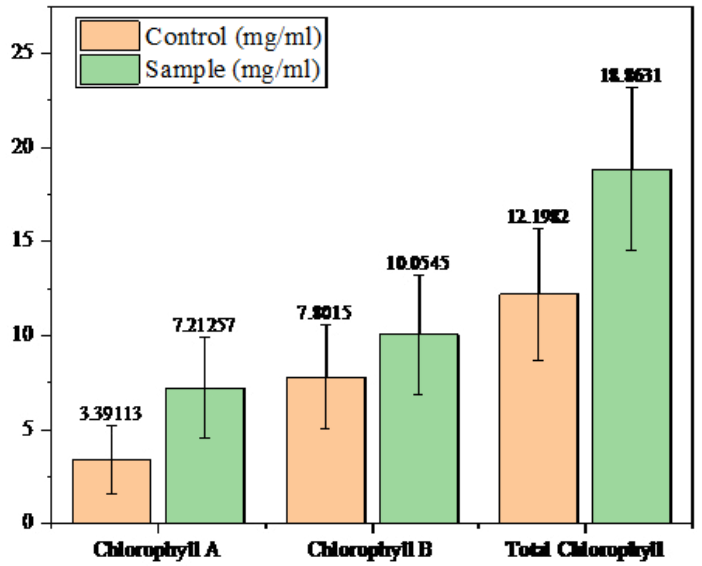


Figure 11. Chlorophyll content comparison of *Vigna radiata*

absorbance values of 1.718 (1 hour), 1.675 (2 hours), and 1.447 (24 hours), demonstrating a slower degradation rate compared to crystal violet. While both CV and MB underwent degradation, among the tested dyes, CV demonstrated a relatively higher percentage of degradation, as shown in Table 4.

GC-MS analysis

The methanolic extract from *Euphorbia geniculata* revealed 40 bioactive compounds, indicating its potential to bear different compounds, as shown in Table 5 and Figure 12. Some of the major compounds are derivatives of 1-butanol, oleic acid, and n-hexadecanoic acid. These findings highlight the broad pharmacological potential of *Euphorbia geniculata*, suggesting its role in possible drug development.

DISCUSSION

In the present study, AgNPs were synthesised by *Euphorbia geniculata.* The plant was chosen based on the reports, which suggested that they are one of the prominent sources of diverse phytocomponents. This chemical diversity acts as efficient reducing and stabilising agents to mediate the production of AgNPs.[48] The noticeable colour transition from pale yellow to reddish brown serves as a preliminary visual confirmation of AgNPs formation. This is well documented with previous reports, which justifies the synthesis process.[49-52] Further, this was substantiated by UV-visible spectroscopy results, which revealed a sharp absorption peak at 418 nm, confirming and characterising the surface plasmon resonance band for AgNPs. This coincides with the AgNPs UV absorbance from the green synthesis protocol.[50-54] Studies report absorbance from 400 to 450 nm, which corresponds to polydispersity and spherical AgNPs.[55,56] Further characterisation using the DLS analysis of AgNPs revealed a polydisperse size distribution with a zeta potential of +0.8 mV. Such a broad size distribution is commonly observed in plant-mediated nanoparticle synthesis, where the complex mixture of phytochemicals adheres to the nanoparticles to make them more stable. Al-Shabib *et al.*[57] reported the production of AgNPs from *Tamarix nilotica* shoot extract, where the DLS analysis showed the polydispersity of the

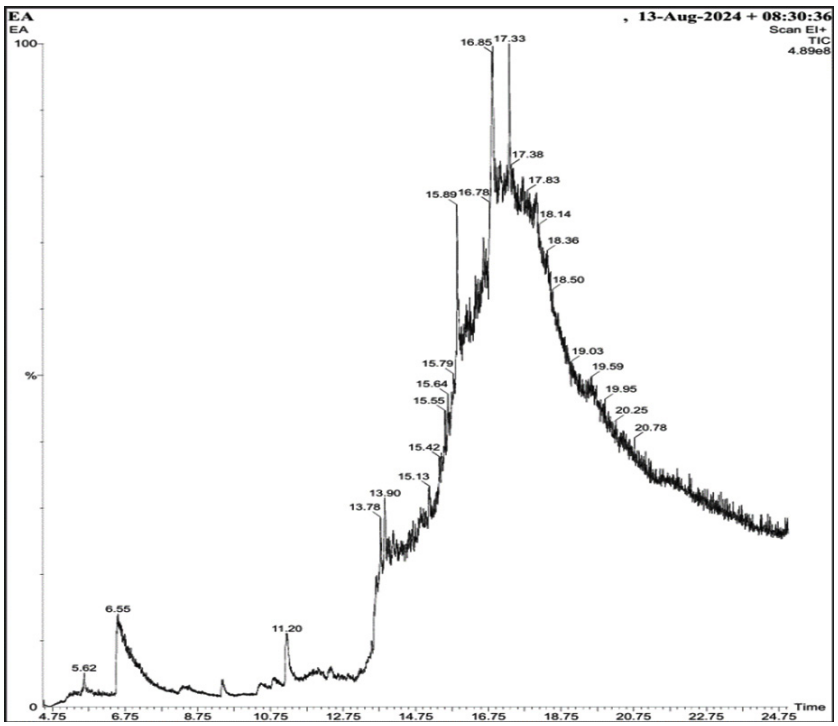


Figure 12. GC-MS Chromatogram of Aqueous Methanol Fraction from *Euphorbia geniculata* Leaves

nanoparticles. This was further confirmed with TEM analysis of AgNPs, which also displayed the polydisperse morphology, with particle sizes ranging from 10 to 100 nm. This observation is consistent with findings from plant-mediated nanoparticle synthesis in a recent scientific report.[57] The FTIR results of AgNPs showed several key functional groups, including hydroxyl (-OH), alkenes (C=C), carbonyl (C=O), alkynes (C≡C), and halogenated groups (-C-Br, -C-C-Cl). These functional groups are indicative of the phytocomponents like terpenoids, saponins, alkaloids, and phenolics present in *Euphorbia geniculata,* which aid in attenuating the desired activity.[58] These functional groups are mainly reported from nanoparticles produced from plant extracts, which confirms the consistency and role of these phytocomponents.[59] The presence of hydroxyl groups has been substantiated in AgNPs synthesized using *Ocimum sanctum*,[60] and the hydroxyl and C=O groups in AgNPs synthesized from *Azadirachta indica*[61] confirmed their role in bio-reduction and surface functionalization. The XRD analysis characteristic peaks were consistently confirmed with previous studies, on biologically synthesised AgNPs, further supporting the crystallinity of AgNPs.[62,63] The peak at 38° is notably intense, indicating a preferential growth orientation, which is a common feature in silver nanoparticle synthesis via plant-mediated reduction methods.[5,64,65] These results corroborate previous findings in green production of nanoparticles, where, unlike chemical and physical production, there might be extracted intensities that might correspond to the presence of phytocomponents as documented in various studies.[64,66,67]

The well-diffusion assay displayed the highest efficacy against *Escherichia coli, Salmonella typhi,* and *Bacillus cereus*, each showed similar inhibition zones of 1.3 cm, while *Staphylococcus aureus* showed only 1.1 cm of inhibition. Thus, AgNPs have broad-spectrum bactericidal properties. The findings are consistent with previous reports of plant-mediated AgNPs,[51,68,69] which exhibited a strong bactericidal effect against both Gram+ and Gram- bacteria. In similar terms, Dutt *et al.*[70] reported bactericidal properties from AgNPs produced from *Azadirachta indica,* which suppressed the proliferation of *E. coli* and *S. aureus*. The mode of action of the nanoparticles is reported, wherein the activity is dependent on size, surface charge, shape, surface coating, agglomeration, solubility, and stability. The smaller size and high surface area of the AgNPs likely enhanced their interaction with bacterial cells, resulting in significant bactericidal effects.[71,72]

The antioxidant capacity of the AgNPs, evaluated using the DPPH radical scavenging assay, revealed substantial activity across varying concentrations (25, 50, and 75 µg/mL). This dose-dependent response aligns with the results of Syed *et al.*[72] The results obtained are in line with the report where *Euphorbia granulata* mediated AgNPs were produced.[73] The results obtained from the studies recommend natural antioxidants against oxidative stress associated with degenerative diseases.[74]

The seed germination and plant growth studies on *Cicer arietinum* and *Vigna radiata* increased the shoot and root measurements, indicating the influence of positive impact of nanoparticles. Additionally, the nanoparticles enhanced chlorophyll content, corroborating the findings of Timi *et al.*[75] that substantiate their role in promoting photosynthetic efficiency and overall plant vitality.

Further, the dye degradation efficiency of AgNPs showed the moderate degradation potential of nanoparticles. The efficiency can be improved by optimizing various parameters, which will be the subject of interest in future studies. The process of degradation aligns with reports of Seerangaraj *et al.,*[76] and Saha *et al.*[77] To barcode the phytoconstituents present in *Euphorbia geniculata*, Soxhlet extraction and GC-MS was carried out which revealed the presence of myriad compounds among which some of the compounds where 1-Butanol, 3-methyl, formate, with notable antimicrobial and aromatic properties[78] oleic acid, known for its antibacterial and antioxidant activities,[79] n-hexadecanoic acid with established anti-inflammatory property[80] antibacterial and antifungal effects,[81] and 9-octadecenamide, (Z), recognized for its anticancer potential.[82,83] The identification of these compounds emphasizes the therapeutic potential of *Euphorbia geniculata*, positioning it as a sustainable source for bioactive agents.

CONCLUSION

The study demonstrates the green synthesis of AgNPs and their potential applications. This approach is efficient, eco-friendly, and cost-effective. It also contributes valuable scientific information on the multifunctional uses of green-synthesized AgNPs. In this study, the AgNPs were well characterized and subjected to a thorough preliminary investigation. Based on the results obtained, future research can focus on optimizing the exact concentration needed for field applications and agricultural greenhouse studies. Further studies can also explore the mechanism behind their antimicrobial properties. In addition, upcoming research may aim to identify the specific phytocomponents responsible for the synthesis and biological activities of the AgNPs.

ACKNOWLEDGMENTS

The authors would like to thank Sri Jayachamarajendra College of Engineering, JSS Science and Technology University, Mysore and Karnataka State Open University, Mysore, for providing the infrastructure for the present study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

SL and SB conceptualized the study. LRM, PA, KGR, SL and HS resource acquisition. SL and VJG performed data curation. MH, LRM and VJG applied methodology. MH performed data investigation. SB and R performed formal analysis. MNNP and KM performed supervision and project administration. SB wrote the original draft. SR, SNR, HS and MNNP wrote, reviewed and edited the manuscript. All authors read and approved the final manuscript for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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