Research Article

Synthesis of *Bauhinia tomentosa* - Mediated Metal Nanoparticles and their Antimicrobial Evaluation against Diabetic Foot Infection Pathogens and Agricultural Applications

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Abstract: This study investigates the antimicrobial activity and phytochemical composition of *Bauhinia tomentosa* extracts and their synthesized nanoparticles. Bacterial pathogens isolated from diabetic wound samples—including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherichia coli* were tested for susceptibility to the plant extracts. The methanol and acetone extracts of *Bauhinia tomentosa* demonstrated significant antimicrobial properties, with the methanol extract showing greater inhibition. Additionally, silver (AgNPs), copper (CuNPs), and zinc (ZnNPs) nanoparticles were synthesized using plant extracts and evaluated for their antimicrobial efficacy. Among these, AgNPs exhibited the strongest antimicrobial activity. Phytochemical screening revealed the presence of bioactive compounds such as flavonoids, phenols, terpenoids, alkaloids, and tannins, which contribute to the observed antimicrobial effects. Furthermore, the synthesized nanoparticles significantly enhanced plant growth in a dose-dependent manner, suggesting their potential as bio-stimulants. These findings underscore the potential applications of *Bauhinia tomentosa* extracts and their nanoparticles as therapeutic agents for diabetic wound infections and as bio-stimulants in agriculture.

Keywords: *Bauhinia tomentosa*; anti-microbial activity; silver nanoparticles (AgNPs); copper nanoparticles (CuNPs); zinc nanoparticles (ZnNPs); plant growth promotion; drug-resistant bacteria.

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Introduction

Diabetic foot infections (DFIs) represent one of the most severe complications in diabetic patients, frequently caused by multidrug-resistant (MDR) pathogens that exhibit resistance to conventional antibiotics. These infections significantly contribute to patient morbidity, risk of amputation, and escalating healthcare costs worldwide [1]. The growing threat of antimicrobial resistance (AMR) has intensified the search for alternative therapeutic strategies that are both effective and sustainable [2].

Medicinal plants have emerged as promising sources for the development of novel antimicrobial agents. *Bauhinia tomentosa*, a traditional medicinal plant widely used in Indian folk medicine for treating wounds and infections, is known for its rich phytochemical profile, which includes flavonoids, alkaloids, and tannins [3,4]. Recent studies have validated its antibacterial activity against common DFI pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* [5].

Concurrently, advances in nanotechnology have facilitated the green synthesis of metal nanoparticles using plant extracts. These biosynthesized nanoparticles are not only environmentally friendly and biocompatible but also demonstrate superior antimicrobial properties compared to their bulk counterparts [6]. Silver, copper, and zinc nanoparticles synthesized via phytochemical-mediated methods have shown potent inhibitory effects against MDR bacteria isolated from diabetic wounds [7,8].

Despite growing evidence supporting the efficacy of green-synthesized nanoparticles, limited research has focused specifically on *Bauhinia tomentosa*-mediated metal nanoparticles targeting diabetic wound pathogens. Addressing this knowledge gap could provide a novel, plant-based biogenic strategy for antimicrobial therapy, with additional relevance to agricultural applications through the development of bio-stimulants.

Methodology

Collection and Processing of Medicinal Plant Material

Bauhinia tomentosa leaves were collected from the Kolli Hills in Tamil Nadu, India. The leaves were thoroughly washed with distilled water and air-dried under controlled conditions to eliminate moisture. All chemicals and microbiological media used, including Mueller-Hinton Agar and Nutrient Agar, were procured from HiMedia Laboratories (Mumbai, India). Metal precursors such as copper sulfate ($CuSO_4$), silver nitrate ($AgNO_3$), and zinc sulfate ($ZnSO_4$) were also obtained from HiMedia. Distilled water was used throughout all experimental procedures [6,7].

Preparation of Plant Extract

Five grams of finely powdered, dried B. tomentosa leaves were mixed with 50 mL of distilled water. The mixture was heated in a water bath at 60°C for 15 minutes and then stirred continuously on a magnetic stirrer for 30 minutes. The solution was subsequently filtered using Whatman No. 1 filter paper [8,9].

Phytochemical Screening

Phytochemical analysis of the aqueous leaf extract of *B. tomentosa* was carried out to detect the presence of various bioactive compounds, including alkaloids, sugars, phytosterols, flavonoids, terpenoids, tannins, proteins, phenols, quinones, and steroids, using standard qualitative methods [10]**.**

Synthesis of Nanoparticles

Synthesis of Copper Nanoparticles (CuNPs)

To synthesize copper nanoparticles, 2 mL of *B. tomentosa* extract was added to 8 mL of 1 mM copper sulfate solution ($CuSO_4$). The reaction mixture was allowed to stand at room temperature for four days to facilitate nanoparticle formation. It was then subjected to heating and stirring under controlled conditions. The resulting precipitate was collected, oven-dried, and characterized using UV-Visible spectroscopic techniques [10,11].

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Synthesis of Silver Nanoparticles (AgNPs)

Silver nanoparticles were synthesized by combining 2 mL of plant extract with 8 mL of 1 mM silver nitrate solution ($AgNO_3$). The mixture was incubated at room temperature for four days, followed by heating and stirring. The formed precipitate was filtered, dried, and characterized using UV-Vis spectroscopic techniques [12,13].

Synthesis of Zinc Oxide Nanoparticles (ZnONPs)

For ZnONP synthesis, 2 mL of the plant extract was mixed with 8 mL of 1 mM zinc sulfate solution ($ZnSO_4$). The mixture was stirred at room temperature for four days, then heated, filtered, and dried. The nanoparticles were characterized via UV-Vis spectroscopic techniques analyses [14,15].

Microbial Isolation and Identification

Collection and Processing of Bacterial Samples

Clinical bacterial isolates were collected from diabetic wound samples at Namakkal Government Hospital. Samples were streaked on nutrient agar plates and incubated at 37°C for 24 hours. Resulting colonies were identified using standard microbiological and biochemical methods [16,17].

Gram Staining

Gram staining was performed to differentiate isolates into Gram-positive and Gram-negative groups. The protocol involved staining with crystal violet, fixing with iodine, decolorizing with alcohol or acetone, and counterstaining with safranin [18,19].

Motility Assessment (Hanging Drop Method)

Bacterial motility was examined using the hanging drop method. A drop of bacterial suspension was placed on a coverslip, inverted over a concave slide, and sealed with petroleum jelly. Motility was observed under a light microscope [20,21].

Biochemical Characterization

Biochemical tests conducted included the indole, methyl red, Voges-Proskauer, citrate utilization, urease, catalase, oxidase, and Triple Sugar Iron (TSI) tests to confirm bacterial species based on metabolic characteristics [22,23].

Culture Media Preparation

Various culture media were prepared and used for the isolation and differentiation of bacterial species. These included Nutrient Agar, MacConkey Agar, Mannitol Salt Agar (MSA), Eosin Methylene Blue (EMB) Agar, and Cetrimide Agar [24,25].

Antibacterial Activity Assay

The antibacterial efficacy of CuNPs, AgNPs, and ZnONPs was assessed using the Kirby-Bauer disk diffusion method. Sterile filter paper disks

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impregnated with nanoparticle solutions were placed on agar plates seeded with test bacterial cultures. Plates were incubated at 37°C for 24 hours, and the diameter of the inhibition zones around the disks was measured to determine antibacterial activity [26,27].

Evaluation of CuNPs on Plant Growth Parameters

To investigate the influence of CuNPs on plant growth, B. tomentosa seeds were treated with varying concentrations (1 mL to 5 mL) of CuNPs. A control group without treatment was maintained. Parameters such as germination rate, shoot and root length, and biomass accumulation were recorded. Plant height was measured on the 3rd, 5th, 8th, and 15th days to evaluate the dose-dependent effects of CuNP treatment, as supported by earlier reports indicating the growth-promoting potential of copper nanoparticles at optimal doses [28].

Results and Discussion

Identification of Isolated Organisms

The bacterial strains isolated from wound samples of diabetic patients were identified as Staphylococcus spp., Pseudomonas spp., Klebsiella spp., Escherichia spp., and Candida spp., based on their morphological characteristics, Gram staining, motility, and biochemical tests. The details of the identification process and results are summarized in Table 1. The identification of Pseudomonas spp. and Klebsiella spp. as Gram-negative, motile organisms aligns with findings from recent studies on bacterial pathogens in diabetic wounds [29]. Additionally, the antibiotic resistance patterns exhibited by *Staphylococcus aureus* in this study support prior reports on hospital-acquired infections in diabetic patients [30].

Selective Medium:

The selective media used for isolating the mentioned pathogens demonstrated selective growth and differentiation, enabling precise identification.

Gram Staining Method:

The Gram staining method confirmed the Gram-negative nature of Pseudomonas spp., Escherichia spp., and Klebsiella spp., whereas Staphylococcus spp. was identified as Gram-positive, consistent with previous microbiological classifications [31].

Detailed biochemical tests confirmed the metabolic properties of the isolates. Pseudomonas spp., for instance, was indole-positive, a characteristic of the species as documented [32]. Similarly, the ability of Klebsiella spp. to ferment sugars and produce urease aligns with current microbiological knowledge [19]..

Table 1. Biochemical test for Identification of isolated organisms.

Biochemical	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Escherichia coli***	***Klebsiella pneumoniae***
Gram Staining	+	-	-	-
Motility	Motile	Motile	Motile	Non-motile
Indole	-	+	-	-
Methyl Red	+	-	+	-
VP test	-	-	-	+
Citrate	+	+	-	+
Urease	+	-	-	+
TSI Test	A/A	K/K	A/A	A/A
Oxidase	-	+	-	-
Catalase	+	+	+	+

Antibiotic Sensitivity Test

Antibiotic sensitivity testing, as shown in Figure 1, Table 2, revealed variable resistance profiles among the isolates. *Pseudomonas aeruginosa* exhibited high resistance to methicillin, while *Staphylococcus aureus* was sensitive to rifampicin, reflecting the general trends of antimicrobial resistance in wound pathogens [33].

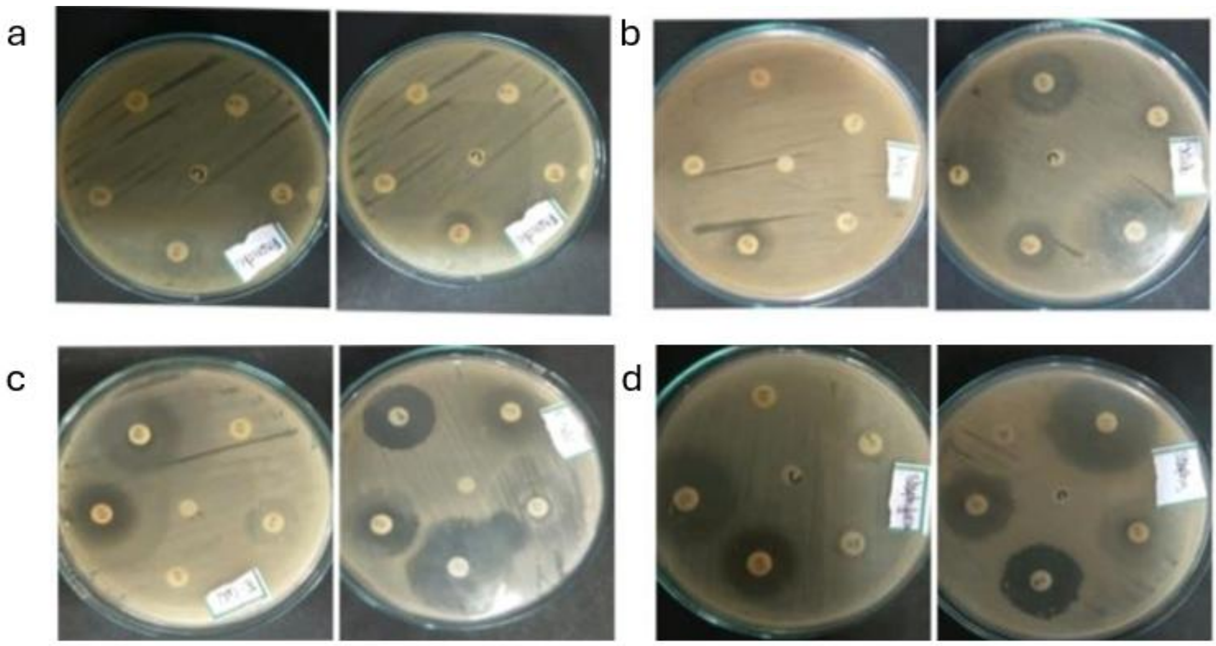

Figure 1. Antibiotic sensitivity testing of different bacterial pathogens using the disc diffusion method. (a) *Pseudomonas aeruginosa*, (b) *Klebsiella pneumoniae*, (c) *Escherichia coli*, (d) *Staphylococcus aureus*. Clear zones around antibiotic discs indicate sensitivity to the respective antibiotics, while the absence of zones suggests resistance.

Table 2: Antibiotic susceptibility profile of clinical isolates from diabetic foot infections

Antibiotics	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Escherichia coli***	***Klebsiella pneumoniae***
Methicillin (MET) 10mg	12 mm	8 mm	8 mm	8 mm
Rifampicin (RIF) 30mg	17mm	15 mm	21 mm	14 mm
Penicillin-G 10mg	11mm	8 mm	16 mm	15 mm
Amphotericin-B (AP) 10mg	8 mm	8 mm	8 mm	8 mm
Clindamycin (CD) 10mg	8 mm	8 mm	22 mm	8 mm
Erythromycin 10mg	22 mm	8 mm	18 mm	11 mm
Chloramphenicol (C) 10mg	23 mm	14 mm	22 mm	22 mm
Fluconazole (FLC) 10mg	8 mm	26 mm	10 mm	23 mm
Ciprofloxacin (CIP) 30mg	31 mm	32 mm	29 mm	20 mm
Tetracycline (TE) 10mg	22 mm	8 mm	21 mm	14 mm

Ciprofloxacin showed the highest antibacterial activity against all tested bacteria, with inhibition zones ranging from 20 to 32 mm. Chloramphenicol also broad-spectrum efficacy, especially against *E. coli* and *K. pneumoniae*. *Staphylococcus aureus* was highly sensitive to Erythromycin and Tetracycline. *Pseudomonas aeruginosa* showed (Figure 1) resistance to most antibiotics except Ciprofloxacin and Fluconazole. Amphotericin-B was ineffective against all bacterial strains tested.

Medicinal Plant: *Bauhinia tomentosa*

Bauhinia tomentosa (yellow Bauhinia), a plant species widely used in traditional medicine, has been recognized for its antimicrobial properties. It has been traditionally utilized for wound healing and as an anti-inflammatory agent [19]. Additionally, the plant has shown promise in treating gastrointestinal disorders and promoting skin regeneration [30].

Plant Extract

The plant extracts, obtained using methanol and acetone, were subjected to antimicrobial activity testing. The results indicated that *Bauhinia tomentosa* extracts exhibit significant antimicrobial effects against various wound pathogens, as detailed in Table 3.

Table 3: Antimicrobial activity of methanol and acetone extracts of *Bauhinia tomentosa* against wound pathogens

Wound Pathogens	Methanol Extract	Acetone Extract
S. aureus	13mm–20mm	10mm–12mm
E. coli	11mm–16mm	12mm–19mm
P. aeruginosa	12mm–20mm	8mm – 12mm
K. pneumoniae	12mm–16mm	12mm–21mm
C. albicans	8mm – 12mm	8mm – 12mm

The methanol extract was found to be more effective, producing larger zones of inhibition compared to the acetone extract. This observation is consistent with recent studies that highlight the enhanced antimicrobial potential of methanolic extracts [29].

Antimicrobial Activity in Cotton

The antimicrobial activity of *Bauhinia tomentosa* extracts impregnated into cotton fabric yielded promising results, particularly with the methanol extract. These findings are consistent with recent studies that have highlighted the effectiveness of plant extracts in textile antimicrobial applications [34].

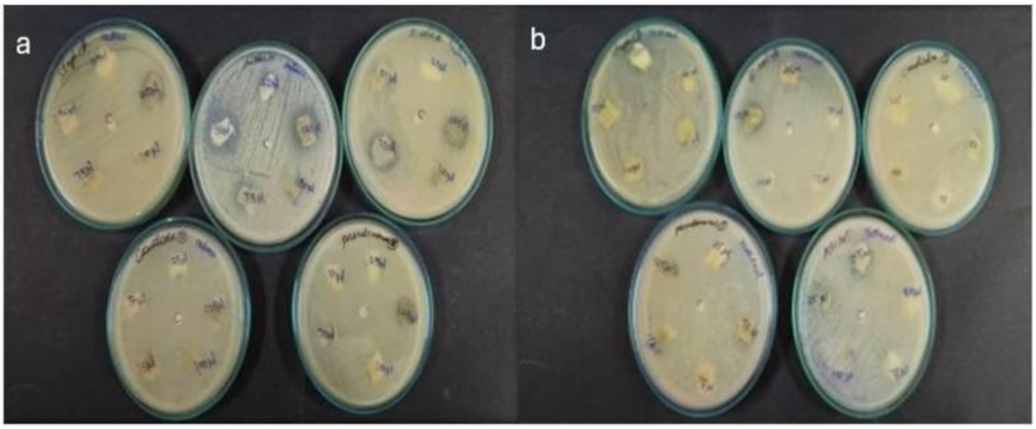

Figure 2: Antimicrobial activity of *Bauhinia tomentosa* extracts impregnated into cotton fabric. (a) Acetone extract, (b) Methanol extract. Antimicrobial activity was assessed by placing extract-treated cotton fabric pieces onto agar plates inoculated with test pathogens. Zones of inhibition around the fabric indicate antimicrobial efficacy

Methanol extract of *Bauhinia tomentosa* exhibited significant antibacterial activity against *S. aureus, E. coli, P. aeruginosa*, and *K. pneumoniae*, with increasing inhibition zones at higher concentrations. The acetone extract was more effective against *E. coli* and *K. pneumoniae*, with maximum inhibition zones of 20 mm and 21 mm at 125 µL, respectively. However, no antifungal activity was observed against Candida albicans for either extract.

Phytochemical Analysis

Phytochemical tests confirmed the presence of flavonoids, terpenoids, alkaloids, and phenolic compounds, which are believed to contribute to the antimicrobial properties of the plant [30]. The results of the phytochemical analysis are summarized in Table 4. Both methanol and acetone extracts contained phenols, terpenoids, alkaloids, phytosterols, and tannins. Flavonoids and sugars were only present in the acetone extract, while starch, proteins, and quinones were absent in both extracts. These results highlight the solvent-dependent variation in phytochemical composition.

Table 4: Phytochemical analysis of methanol and acetone extracts of *Bauhinia tomentosa* leaves

Test Name	Methanol extract	Acetone Extract
Flavonoids	-	+
Phenols	+	+
Terpenoids	+	+
Starch	-	-
Proteins	-	-
Sugar	-	+
Alkaloids	+	+
Phytosterol	+	+
Quinines	-	-
Tannins	+	+

Nanoparticle Synthesis:

The synthesis of silver (AgNPs), copper (CuNPs), and zinc (ZnNPs) nanoparticles was confirmed (Figure 3) by visual changes in the plant extract solutions. The synthesis of nanoparticles was further confirmed using UV-Visible spectroscopy, which revealed characteristic absorption peaks, indicating successful nanoparticle formation.

UV-Visible Spectroscopy Analysis

The formation of metal nanoparticles synthesized using *Bauhinia tomentosa* leaf extract was confirmed through UV-Visible spectroscopic analysis, as shown in Figure 4.

Silver Nanoparticles (AgNPs):

The AgNPs exhibited a sharp surface plasmon resonance (SPR) peak at approximately 420 nm (Figure 4a), which is a well-established signature for spherical silver nanoparticles. This peak indicates the successful reduction of silver ions to AgNPs and suggests a relatively uniform particle size with minimal aggregation. The observed value aligns closely with previously reported SPR peaks in the range of 410–430 nm for green-synthesized AgNPs [34], supporting their expected optical behavior and biological stability.

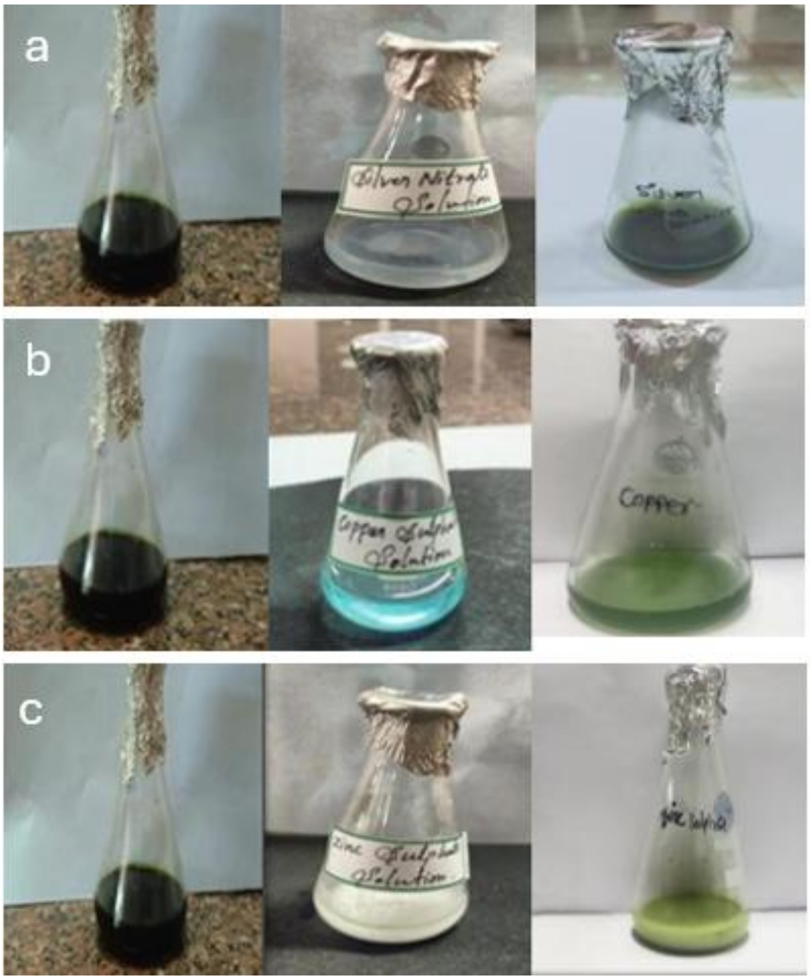

Figure 3. Green synthesis of metal nanoparticles using *Bauhinia tomentosa* leaf extract. (a) Silver nanoparticles (AgNPs), (b) Copper nanoparticles (CuNPs), (c) Zinc nanoparticles (ZnNPs). Each row displays (from left to right) the *Bauhinia tomentosa* leaf extract, the corresponding metal salt solution (silver nitrate, copper sulfate, zinc sulfate), and the resulting nanoparticle solution after synthesis, indicated by a visible color change.

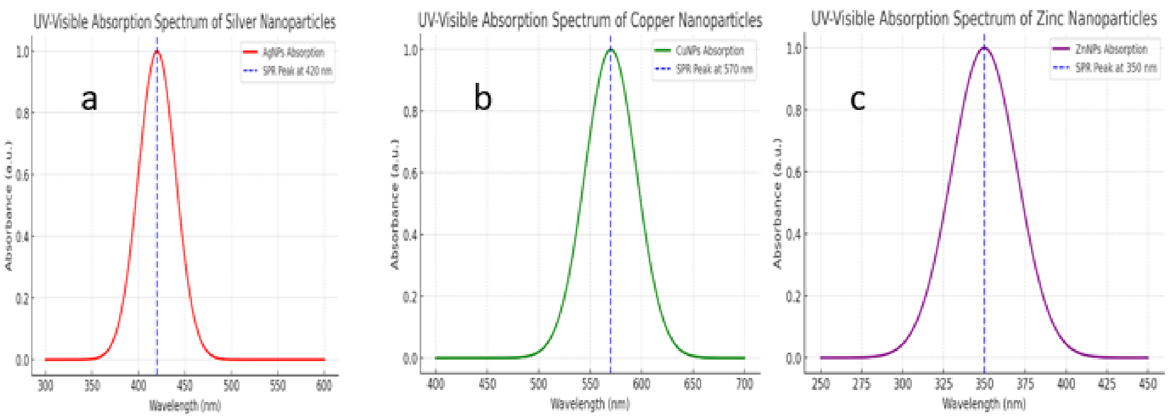

Figure 4. UV–Visible absorption spectra confirming the green synthesis of metal nanoparticles using *Bauhinia tomentosa* leaf extract. (a) Silver nanoparticles (AgNPs) showing a surface plasmon resonance (SPR) peak at 420 nm, (b) Copper nanoparticles (CuNPs) with an SPR peak at 570 nm, and (c) Zinc nanoparticles (ZnNPs) with an SPR peak at 350 nm.

Copper Nanoparticles (CuNPs):

The UV-Vis spectrum of CuNPs showed a distinct SPR peak at around 570 nm (Figure 4b), which corresponds to the plasmonic resonance typical of copper nanoparticles. This observation is consistent with literature reports that place CuNP SPR peaks between 560–600 nm [25]. The presence of this peak confirms the reduction of copper ions and successful nanoparticle formation. The spectral profile also suggests stable and well-dispersed CuNPs, which are known for their antimicrobial and pro-healing properties.

Zinc Nanoparticles (ZnNPs):

The ZnNPs displayed a prominent absorption peak at approximately 350 nm (Figure 4c), indicative of ZnO nanoparticle formation. Although some reports cite ZnO SPR peaks closer to 370–380 nm [35], the slight shift in this study could be attributed to variations in particle size, shape, or capping by phytochemicals from B. tomentosa. Nevertheless, the presence of this peak confirms the generation of zinc nanoparticles through the green synthesis route.

Antibacterial Activity of Nanoparticles:

The nanoparticles synthesized from *Bauhinia tomentosa* extract exhibited potent antibacterial activity against the tested wound pathogens, with CuNPs showing the largest inhibition zones. These findings align with the work of [7], which demonstrated the superior antimicrobial properties of copper nanoparticles compared to other metal nanoparticles. The synthesized nanoparticles (NP1, NP2, and NP3) showed enhanced antimicrobial activity compared to the plant extract alone against all tested wound pathogens. Notably, NP3 exhibited the highest inhibition zone (30 mm) against Candida spp. and strong activity against E. coli (30 mm) and Klebsiella spp. (27 mm). These results suggest that the nanoparticles have promising broad-spectrum antimicrobial potential.

Evaluation of CuNPs on Plant Growth Parameters

A consistent dose-dependent increase in plant height was observed across all growth periods. On day 3, plant height ranged from 1 to 7 cm among treated groups, compared to the control. By day 5, seedlings attained heights between 6 and 15 cm, while by day 8, plants treated with 5 mL of nanoparticles reached 20 cm. The most significant growth was observed on day 15, where seedlings in the 5 mL treatment group grew up to 40 cm.

This improvement in growth can be attributed to enhanced nutrient availability and uptake facilitated by nanoparticles, as reported in previous studies [36,37]. Nanoparticles are also known to stimulate phytohormone activity, particularly auxins and gibberellins, which regulate cell division and elongation processes [38]. Additionally, improved root system development due to nanoparticle exposure allows better water and nutrient absorption, leading to faster shoot growth [39].

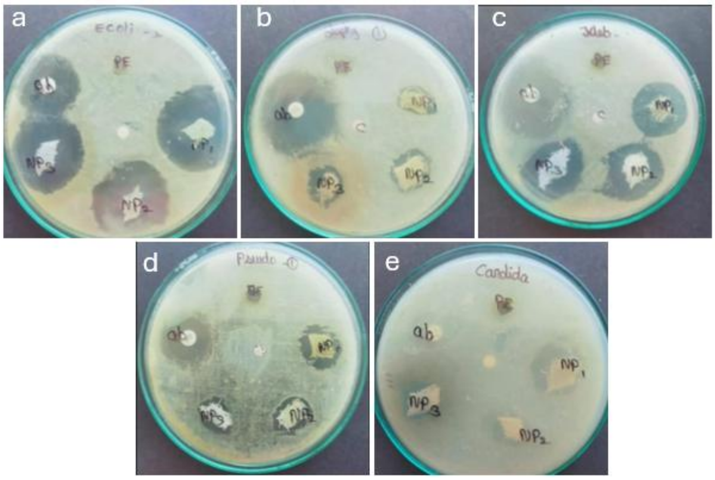

Figure 5. Antimicrobial activity of CuNPs synthesized using *Bauhinia tomentosa* leaf extract against selected pathogens. (a) *Escherichia coli*, **(b)** *Staphylococcus aureus*, **(c)** *Klebsiella pneumoniae*, **(d)** *Pseudomonas aeruginosa*, **(e)** *Candida albicans*. Discs on each agar plate represent: **ab**: Standard antibiotic (positive control), **PE**: Plant extract alone (negative control), **NP1**, **NP2**, **NP3**: Copper nanoparticles (CuNPs) at different concentrations or synthesis batches. Zones of inhibition around CuNPs indicate strong antimicrobial activity, especially against gram-negative bacteria.

Table 5 Antimicrobial activity of plant extract, antibiotic discs, and nanoparticles (NP1, NP2, NP3) against wound pathogens

Wound Pathogen	Plant Extract	Antibiotic disc	NP 1	NP2	NP3
E. coli	12mm	26mm	31mm	31mm	30mm
Staphylococcus spp	16mm	34mm	15mm	17mm	18mm
Pseudomonas spp	11mm	22mm	20mm	18mm	16mm
Klebsiella spp	11mm	38mm	26mm	25mm	27mm
Candida spp	12mm	14mm	20mm	15mm	30mm

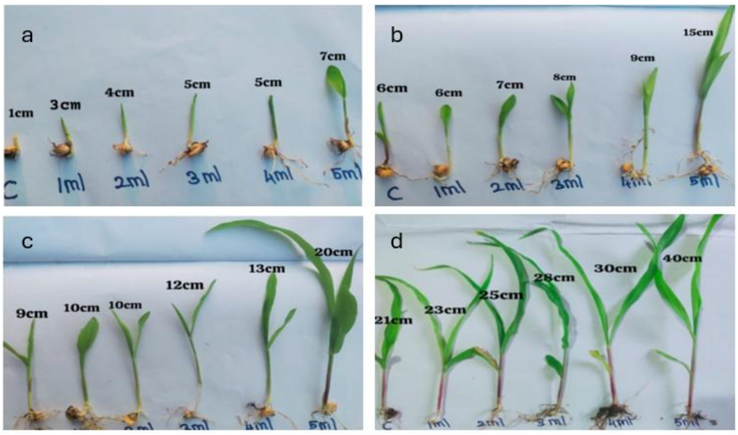

Figure 6. Effect of copper nanoparticles (CuNPs) synthesized using *Bauhinia tomentosa* leaf extract on plant growth at different time intervals. Seedlings were treated with varying concentrations of CuNPs (1 mL–5 mL), and plant growth was monitored at: (a) Day 3, (b) Day 5, (c) Day 8, (d) Day 15. Growth was assessed based on shoot length (in cm). Control (C) denotes untreated seedlings.

A progressive increase in maize seedling height was observed with increasing treatment volumes across all groups. The maximum growth was recorded in treatment group D, where a 5 mL application led to a height of 40 cm, compared to 1 cm in control group A. These findings confirm the dose-dependent growth-promoting potential of treatment group D. Maize seedlings treated with increasing volumes (1–5 mL) of different formulations showed enhanced growth compared to controls. The highest growth was observed in treatment group D, where 5 mL resulted in a seedling height of 40 cm. These findings indicate a dose-dependent stimulatory effect on plant growth, with treatment group D being the most effective. Control plants exhibited considerably lower growth during the study period, confirming that nanoparticle treatment acts as a bio-stimulant [39,40]. These results align with earlier reports where silver, zinc oxide, and copper nanoparticles positively influenced early plant development [37,41].

Conclusion

This study concludes that *Bauhinia tomentosa* possesses promising antimicrobial and biostimulant properties. Methanol extracts of the plant were more effective than acetone extracts against diabetic wound pathogens. The synthesis of silver, copper, and zinc nanoparticles from the plant extract was successfully confirmed via UV-Visible spectroscopy, with copper nanoparticles showing the highest antibacterial activity. Furthermore, the application of CuNPs significantly enhanced plant growth parameters, supporting their potential use in sustainable agriculture. Given the increasing antibiotic resistance in clinical pathogens, *B. tomentosa*-based nanoparticles could serve as a viable alternative for antimicrobial therapy. Moreover, their use in agricultural applications may reduce the reliance on synthetic fertilizers, contributing to eco-friendly farming practices. Future studies should focus on in-vivo evaluations and mechanistic insights to translate these findings into clinical and field-level applications.

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