

Development and Assessment of Copper-Based Nanoparticles Derived from *Dypsis lutescens* for Their Antibacterial Properties by *in vitro* Application

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Abstract:

Objective: Phytoconstituents, including polyphenols, tannins, alkaloids and flavonoids, which are abundant in *Dypsis lutescens* leaf extract, were utilized for the green synthesis of copper nanoparticles (g-CuNPs). These bioactive compounds served as natural reducing agents and capping agents in the synthesis process.

Material and Methods: The formation of g-CuNPs was confirmed by ultraviolet spectroscopy (UV), showing a characteristic λ_{max} at 410 nm. Functional groups of the capping agents on g-CuNPs were verified using Fourier transform infrared spectroscopy (FTIR spectroscopy).

Results: The nanoparticles demonstrated remarkable stability, as confirmed by Malvern Zetasizer analysis. They exhibited an acceptable particle size and Poly Dispersity Index (PDI), along with a robust positive zeta potential of +40, indicating their suitability for biological applications. Antibacterial assays revealed that the g-CuNPs significantly enhanced antibacterial activity compared to the leaf extract alone. The inhibition zones were slightly higher for both Gram-positive and Gram-

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negative bacteria, including *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus* (*S. aureus*) and *Enterococcus faecalis* (*E. faecalis*), underscoring the efficacy of the nanoparticles in combating bacterial pathogens.

Conclusion: This study underscores the potential of integrating bioactive phytochemicals with copper nanoparticles (CuNPs) to create potent antibacterial agents. The enhanced activity of g-CuNPs against both Gram-positive and Gram-negative bacteria highlights their promise as effective solutions for addressing challenges posed by pathogenic bacteria. This innovative approach paves the way for developing advanced antimicrobial therapies with improved efficiency and stability.

Keywords: antibacterial activity, copper nanoparticles (CuNPs), *Dypsis lutescens*, green synthesis

Introduction

In recent years, traditional medicine has gained popularity due to the country's prolonged and unsustainable economic position, which has been exacerbated by the exorbitant prices of pharmaceuticals and the increasing prevalence of medication resistance to prevalent illnesses. The therapeutic approach has shifted to alternative traditional medicine in order to conduct a concerted search for new chemical entities. Herbal remedies are extensively utilized in traditional medicine and their therapeutic capacities are firmly established¹. The adverse effects can be reduced when compared to conventional methods. Traditional medicine refers to a collection of practices, systems, knowledge and beliefs concerning health that encompass manual treatments, exercises, spiritual cures and medications obtained from plants, animals and minerals. These methods can be used alone or in combination to treat, diagnose and prevent diseases and preserve health. When embraced outside its traditional culture, traditional medicine is frequently called "complementary and alternative medicine." Today's most extensively utilized traditional medicine systems are those of China, India and Africa².

A nanoparticle, or an ultrafine particle, is a matter particle with a dimension of 1 to 100 nanometers. Nanoparticles (NPs) possess unique characteristics,

including a high surface area-to-mass ratio, ultra-small size and enhanced reactivity, which contribute to their modified properties. Extensive research has been conducted on metal nanoparticles due to their diverse applications in catalysis, medicine, pharmaceuticals and agriculture. The word is sometimes used for bigger particles (up to 500 nm)³. Nanoparticles have several applications in various fields, including the environment, agriculture, food, biotechnology, medicine and pharmaceuticals⁴. Modern advances in nanoscience and technology have occurred recently, primarily due to the increased use of nanoparticles because of their exceptional quality, effectiveness and inventiveness. Nanoparticles can be synthesized using physical, chemical and biological approaches. Physical and chemical methods often yield low quantities and cause various toxicity issues as harmful chemicals are used during synthesis. This has led to a shift to a biological approach, which produces more quantity and can be produced rapidly and is cost-effective, non-toxic and biodegradable. Regarding the biological method, various sources such as plants, bacteria, fungi, yeast and algae are utilized. Plant-based is often recommended as it gives more yield and is non-toxic. It has been reported that plant-based products have had good antibacterial results hitherto⁵. The green manufacturing of metal nanoparticles

is a low-cost, plant-mediated, physiologically active and environmentally benign process⁶. Usually measuring between 1 and 100 nanometers, metallic nanoparticles are minuscule particles of metal atoms. Because of their small size and high surface area-to-volume ratio, these nanoparticles have unique physical, chemical and electrical characteristics. They can be made from copper, platinum, silver and gold⁷. Their diverse applications include optics, catalysis, optoelectronics, chemical/biochemical sensing, biomedical and nanostructure fabrication. The use of plant extracts in synthesizing metal nanoparticles is of great interest because it exhibits dual functionality as both a reducing and capping agent⁸. Researchers increasingly use green synthesis methods for metal nanoparticles to meet the growing demand for environmentally friendly properties^{9,10}. Copper is the most widely used material in the world due to its electrical, optical, catalytic, biomedical and antifungal/antibacterial applications; with gold and silver, copper nanoparticles act as antimicrobial agents in various fields¹¹. It is safe for human use in food packaging and water treatment^{12,13}.

The *Areca palm* is a multipurpose, evergreen, perennial tree. It is a member of the Arecaceae family. In India, it is one of the most commonly used addictive substances for chewing, right behind nicotine and tobacco worldwide. The *Areca palm* is essential for its therapeutic properties, which include antibacterial, antimicrobial, anti-inflammatory, antioxidant, and so on. The *Areca palm* and its nuts, leaves, and roots, among other parts, have strong antibacterial properties. *Areca nut* extract is effective against Gram-positive and Gram-negative bacteria¹⁴. Alkaloids, tannins, flavones, triterpenes, steroids, and fatty acids are among the more than 59 compounds that have been separated and identified from *A. catechu*. *A. catechu*-derived compounds and extracts exhibit a variety of pharmacological actions. Copper nanoparticles (CuNPs) exhibit strong antidepressant, antibacterial,

anti-inflammatory, and antioxidant activities, according to previous studies¹⁵. Leaves of this plant have been used as a tonic, cordial, and diaphoretic. Studies have revealed that it possesses antibacterial, antifungal, and analgesic properties. Previous studies on palm species suggest that they contain bioactive compounds, such as flavonoids, tannins, alkaloids, saponins, and phenolics, which exhibit antibacterial properties. Investigating the phytochemical constituents of *Dyopsis lutescens* through extraction and analysis can provide insights into its potential antimicrobial efficacy. A study by Aamir Ejaz et al. performed antibacterial studies using *Cyperus scariosus* that were done by coating nanoparticles with gold, indicating the zone of inhibition for the CSRE compound of *E. coli* (10 ± 0.2) with that of CSRE@AuNPs (18 ± 0.3). Hence, coated particles were found to be more active than uncoated nanoparticles¹⁶.

The antimicrobial potential of these natural bioactive compounds present in the extract of plants and can be enhanced by surface coating the small sized particles that have a large surface area, such as CuNPs, as they can easily cross the membrane barriers inside the cell, due to being micron sized, and exhibit the synergistic role of NPs; and, bioactive compounds can inhibit the growth of microorganisms more effectively. Contrastingly, due to the presence of polyphenols, the extract can be easily reduced to CuNPs from Cu^{2+} under normal conditions when stirring in the presence of sunlight. Therefore, the leaf extract of *Dyopsis lutescens* was utilized to synthesize and stabilize CuNPs.

Metal nanoparticles (NPs) serve as efficient photocatalysts for dye degradation under sunlight by facilitating electron excitation from the valence to the conduction band, generating electron-hole pairs that drive redox reactions. Their photocatalytic efficiency depends on factors like size, shape, and bandgap energy, which can be enhanced by modifying, reducing, and capping agents. Plant-based NP synthesis is an emerging field of

interest. Additionally, copper nanoparticles (CuNPs) exhibit biological activity, penetrating cells and inducing reactive oxygen species that disrupt protein function and genetic material, leading to cell death. Hence, CuNPs can also be used in biological applications to inhibit the bacterial growth responsible for various diseases in humans.

In this research article, we aimed to synthesize CuNPs using the leaf extract of *Dyopsis lutescens* and explore the potential application in antibacterial studies. The synthesized NPs were characterized using Ultraviolet-Visible Spectroscopy (UV-vis), Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), and Dynamic Light Scattering (DLS) in antibacterial studies¹⁷. Infectious diseases are a prominent contributor to global mortality, and the issue of antibiotic resistance has emerged as a problem of worldwide significance. Using new compounds based on antimicrobial, antioxidant, hemolytic, and cell line studies can help prevent antibiotic resistance in pathogens¹⁸.

Material and Methods

Plant sample collection and authentication:

The *Dyopsis lutescens* leaf was collected from Pilikula, Mangalore, in September and October and further authenticated by Alva's Institute, Moodbidri, Mangalore, Karnataka. The leaf's surface was cleaned and washed using tap water, followed by distilled water to eliminate dust particles, and dried in the shade for 15 days to remove moisture from the leaves. The dried leaves were finely powdered and stored in a clean, airtight container.

Preparation of plant extract:

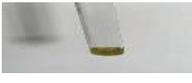
The extraction was carried out by a Soxhlet process: 50 g of powdered plant material was packed into a thimble and extracted with 400 ml of methanol with a single solvent. The extraction was continued at 60 °C till the solvent in the siphon tube of an extractor became colorless. The

extract obtained was then transferred to a china dish and kept in an electric water bath at 30–40 °C until the solvent evaporated¹⁹.

Preliminary phytochemical screening:

Chemical tests were conducted on extracts to detect alkaloids, glycosides, phenolic compounds, flavonoids, flavones, terpenoids (Table 1), and sterols²⁰.

Table 1 Results of preliminary phytochemical investigation

Sl. No.	Phytoconstituents	Methanolic leaf extract (photos and results)	
1	Alkaloids		+ve
2	Flavonoids		+ve
3	Diterpenes and Terpenoids		+ve
4	Proteins		+ve
5	Phenols		+ve

Green synthesis of copper nanoparticles (CuNPs) and copper oxide nanoparticles from *Dyopsis lutescens* leaves

A 0.2 M solution of $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ in water was prepared and kept in brown bottles. To this, 100 ml of plant leaf extract was combined with 400 ml of the 0.2 M $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ solution at a 1:4 ratio, adding it drop by drop while continuously stirring. The mixture underwent a 24-hour incubation period at an ambient temperature. The color change was examined at regular intervals (after 30 and 60 minutes). The color change from blue to light brown

indicated the creation of Cu NPs. The solution was then centrifuged for 15 minutes at 10,000 rpm. The produced g-Cu NPs were washed with deionized water and ethanol to eliminate contaminants. The CuNPs were then allowed to dry and grind before being used for further examination (Figure 1)²¹.

Characterization of green-synthesized copper nanoparticles (CuNPs)

The following techniques determined the morphology of CuNPs:

UV-visible spectroscopy:

It is a widely used technique for characterizing copper nanoparticles. Copper nanoparticles exhibit a characteristic surface plasmon resonance (SPR) peak in the 200–800 nm range. The position and intensity of this peak can provide information about the size and concentration of the nanoparticles. It is a widely used technique for characterizing copper nanoparticles. Copper nanoparticles exhibit a characteristic surface plasmon resonance (SPR) peak in the 400–800 nm range. The position and intensity of this peak can provide information about the size and concentration of the nanoparticles. Shimadzu's UV-2600

spectrophotometer measured the samples' UV-visible absorbance and reflectance spectra between 200 and 800 nm. While UV-DRS stands for diffuse reflection spectroscopy, transmission was used to measure UV-visible spectra. Following sufficient dilution, the newly produced plant extract solution was mixed with the copper solution to make the copper nanoparticle solution. The absorbance spectrum was recorded by scanning wavelengths from 200 to 800 nm. The absorbance of the nanoparticle solution was compared to the blank extract solution²².

Fourier transform infrared spectroscopy (FTIR)

is employed to study the surface chemistry of copper nanoparticles. It helps identify functional groups of stabilizing or capping agents on the nanoparticle surface. FTIR spectra can provide insights into the interactions between copper nanoparticles (CuNPs) and stabilizing agents. It is employed to study the surface chemistry of CuNPs. It helps identify functional groups of stabilizing or capping agents on the nanoparticle surface. FTIR spectra can provide insights into the interactions between CuNPs and stabilizing agents. A highly efficient technique for analyzing the chemical content and structure of materials, including small particles like copper nanoparticles, quantifies the

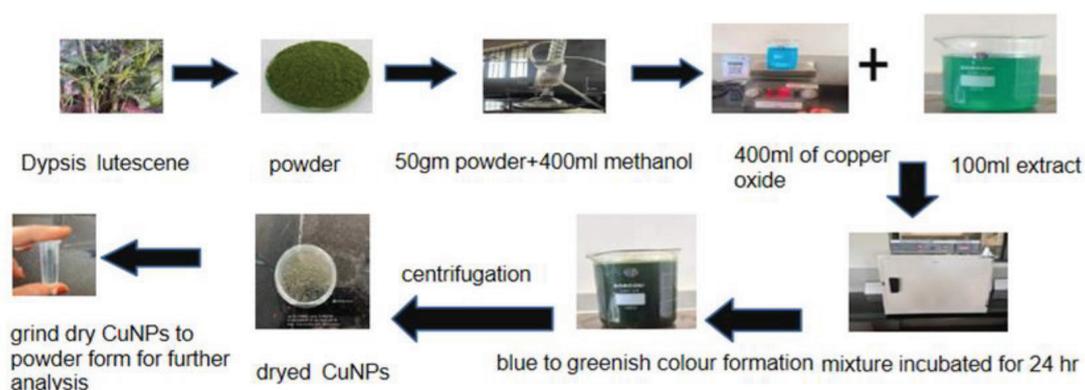


Figure 1 Schematic representation of the synthesis of CuNPs from *Dypsis lutescens* leaf

absorption of infrared light for the sample by varying the laser wavelength. Different chemical bonds absorb infrared radiation at specific wavelengths, providing information about the functional groups present in the sample. An FTIR instrument consists of a source of infrared radiation, a sample compartment, a detector, and an interferometer. The interferometer modulates the infrared radiation, and a computer processes the resulting interferogram to generate a spectrum. For analysis of copper nanoparticles, the nanoparticles are typically dispersed in a suitable solvent or matrix to form a thin film or pellet. This ensures uniformity and facilitates the measurement of the sample. FTIR can identify functional groups on the surface of copper nanoparticles. If the copper nanoparticles are modified or functionalized, FTIR can provide information about the nature of these modifications. FTIR can also monitor copper nanoparticle chemical reactions (Figure 2)²³.

Dynamic light scattering (DLS):

DLS studies were conducted to confirm the nano size, particle size, particle size distribution, zeta potential,

and polydispersity index (PDI). The Malvern Zetasizer integrates 3 techniques into one compact device. It utilizes DLS to measure particle size, leveraging the Stokes–Einstein correlation to determine the size and distribution of particles in motion due to Brownian motion. Zeta potential was assessed using laser Doppler microelectrophoresis, where an electric field is applied to a solution or particle dispersion, resulting in particle movement that is directly proportional to their zeta potential. An accessible zeta potential accessory employs tracer particles to assess electro-osmosis near a sample's surface, facilitating the calculation of the surface's zeta potential (Figure 3)²⁴.

SEM analysis:

The CuNPs were dried at 60 °C to obtain a powdered form and characterized using field emission scanning electron microscopy (FESEM, FEI-Quanta FEG 650), transmission electron microscopy (TEM), energy dispersive spectroscopy (EDS), X-ray diffraction (XRD, PANalytical Japan), and Fourier–transform infrared spectroscopy (FTIR, Shimadzu 8400S).

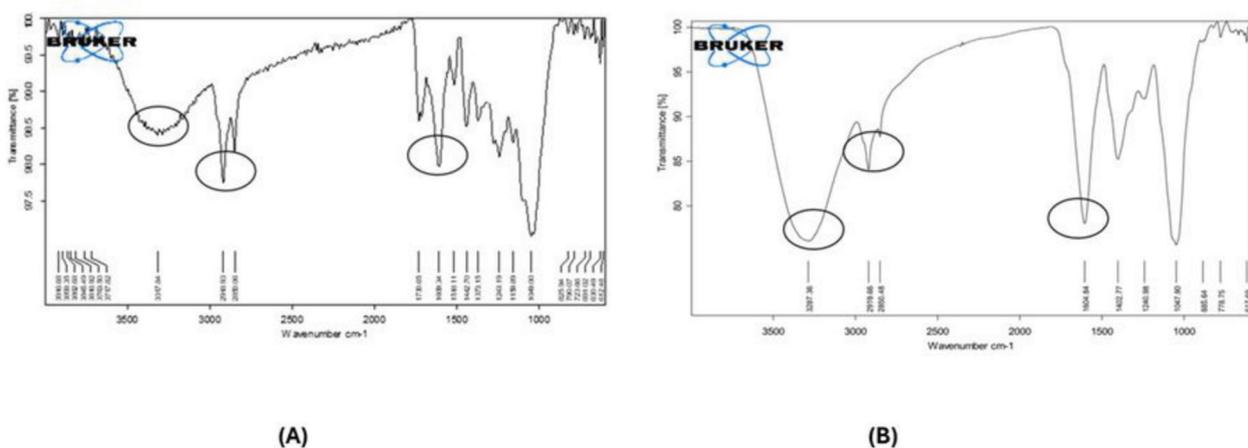


Figure 2 (A) FTIR of CuNPs from *Dypsis lutescens* leaf extract (B) FTIR of synthesized plant extract from *Dypsis lutescens*

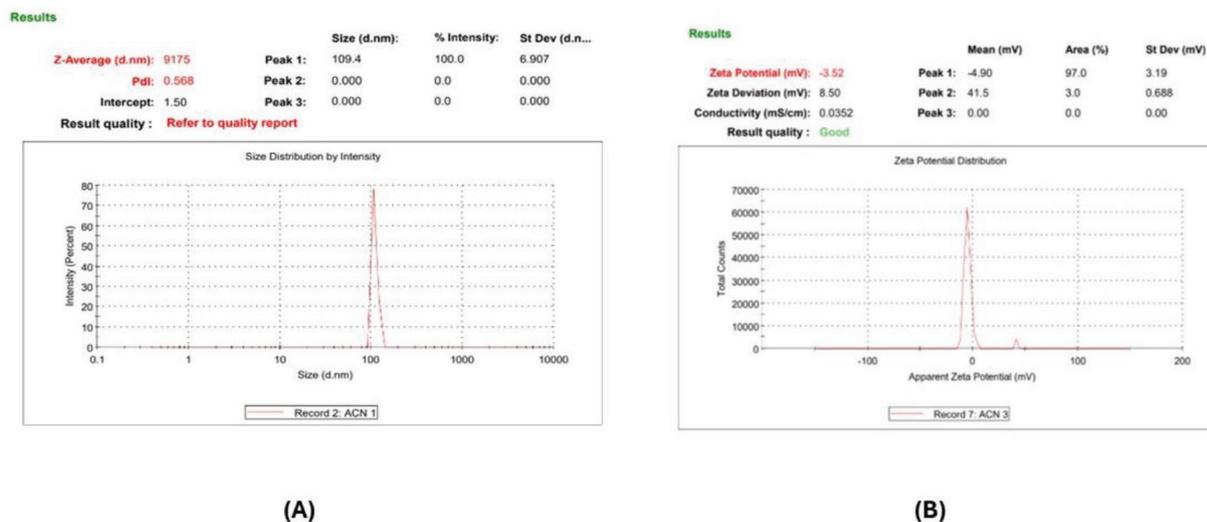


Figure 3 (A) Particle size distribution of synthesized CuNPs from *Dypsip lutescens* leaf extract and (B) Zeta potential of synthesized CuNPs *Dypsip lutescens* leaf extract–tract

Screening of *in vitro* antibacterial activity

The samples were individually tested against 2 Gram-negative bacteria, *Escherichia coli* (MCC25175) and *Pseudomonas aeruginosa* (ATCC27853), and 2 Gram-positive bacteria, *Staphylococcus aureus* (MCC2408) and *Enterococcus faecalis* (ATCC 29212). Antibacterial activity was assessed following the CLSI Guidelines. To assess the antibacterial efficacy of the test samples, we used agar disc diffusion technology. Microbial suspensions with a turbidity equivalent to a McFarland 0.5 standard and then diluted to 105 cfu/mL with sterile distilled water. The adjusted microbial suspensions (100 μ L) were spread onto the Muller-Hinton agar plates. Subsequently, test samples were placed. Gentamicin (10 mcg) was used as a positive control against *Escherichia coli* and *Pseudomonas aeruginosa*, and Vancomycin (30 mcg) was used as a positive control against *Staphylococcus aureus* and *Enterococcus faecalis*. Plates were kept at 37 °C for 24 hours. The diameters of clear inhibition zones were measured using a caliper to evaluate the samples' antimicrobial potential²⁵.

Results

The percentage yield of the extraction:

The extraction of plant leaf material was carried out using 50 g of powdered plant material. After the extraction process, the yield of the extract obtained was 3.51 g. Thus, the extraction process resulted in a 7.02% yield of the plant extract, following the active-constituent alkaloids, flavonoids, diterpenoids, terpenoids, and phenols, which were found positive, as mentioned in Table 1. This yield indicates the successful recovery of bioactive constituents from the plant material for further analysis and applications. The yield of methanol extract after Soxhlet extraction was found to be 7.02%

Qualitative analysis of major phytochemicals in both extracts:

Below is the qualitative analysis of the phytochemicals in both extracts. Methanol extract was rich in flavonoids, alkaloids, and phenolics, whereas aqueous extract contained more tannins, saponins, and carbohydrates (Supplementary Table 1).

Characterization of CuNPs by UV:

UV-Vis spectroscopy at 410 nm is commonly used to analyze copper nanoparticles (CuNPs) based on their surface plasmon resonance (SPR). A sharp peak indicates monodisperse nanoparticles with uniform size, while a broad peak suggests polydispersity with varying particle sizes. Peak shifts can indicate size changes or aggregation, affecting nanoparticle stability and functionality.

Characterization of CuNPs by FTIR

FTIR spectroscopy of our CuNPs revealed distinctive bands at 3,374, 2,918 and 1,730 cm^{-1} , which confirmed the presence of -OH str, -C-H str, -C=O str. Synthesized plant extract showed peaks at 3,287, 2,918 and 1,604 cm^{-1} , confirming -NH str, -C-H str, C=C str, which confirmed the presence of copper in the formulation.

Characterization of CuNPs by DLS

Particle size, zeta potential, and PDI of CuNPs were analyzed using MALVERN ZETASIZER. The particle size for CuNPs was 109.4 nm; zeta potential was found to be

-4.90 mV. PDI was found to be 0.568%, which confirmed the stability of nanoparticles due to electrostatic interaction. The polydispersity index (PDI) measures the size distribution of nanoparticles, where a lower PDI (<0.3) indicates uniformity and higher stability, while a higher PDI (>0.3) suggests polydispersity and potential aggregation. Stable nanoparticles typically have a low PDI, ensuring better dispersion, reduced agglomeration, and improved functional performance in biological and industrial applications. They can remain stable for weeks to months, depending on factors like pH, ionic strength, and stabilizers. Without stabilizers, oxidation and sedimentation may occur within a few weeks, reducing their effectiveness (Figure 3).

SEM analysis:

The morphology of CuNPs was analyzed using FESEM, revealing that the synthesized nanoparticles were spherical with a tendency to form random aggregates. However, CuNPs synthesized through the green synthesis process exhibited a more defined shape with reduced aggregation, indicating improved dispersion (Figure 4).

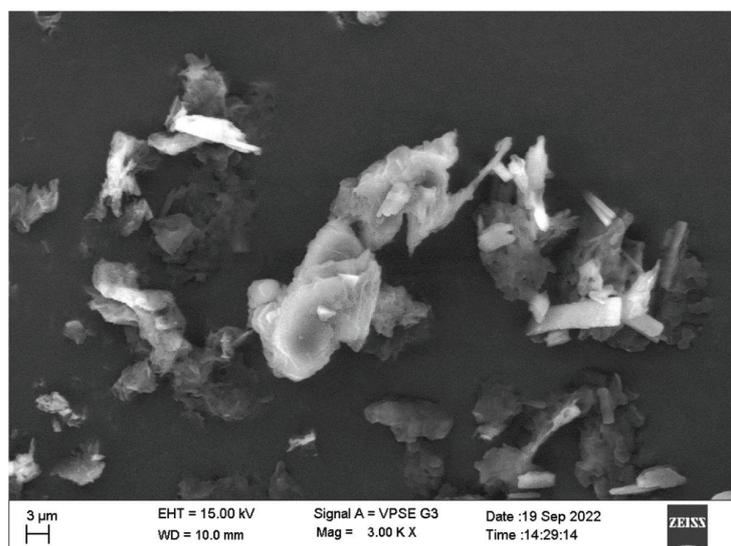


Figure 4 SEM image of CuNPs of *Dypsis lutescens* leaf extract

In vitro antibacterial studies

The *in vitro* antibacterial activity of CuNPs was evaluated using the agar disc diffusion method against 2 Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and 2 Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*), in Figure 5. Gentamicin (10 mcg) served as the positive control for Gram-negative bacteria, while Vancomycin (30 mcg) was used for Gram-positive bacteria. Clear inhibition zones were observed, indicating the significant antibacterial activity of the CuNPs.

For *E. coli*, a comparison was made between extract and synthesized compounds, which showed an inhibition of 16% compared to the extract (6%). Similarly, it was compared with other bacteria and showed good inhibition (12, 13 & 18%); see Table 2. From the previous research study conducted by Mohammad et al. (2019), it was reported that CuSO₄ nanoparticles were performed on HUVEC

cell lines and reported that upon inducing doses of up to 1,000 µg/ml, the cells were viable, indicating that there was no toxicity upon the heavy administration of doses, and thus showing its safety in clinical applications. (Novel synthesis of *Falcaria vulgaris* leaf extract conjugated copper nanoparticles with potent cytotoxicity, antioxidant, antifungal, antibacterial, and cutaneous wound healing activities under *in vitro* and *in vivo* conditions). A study conducted by Shabnam Amin et al. (2022) reported that bacterial strains conducted on *B. Subtilis*, *S. Typhi*, *S. Aureus*, and *E. Coli* have shown zones of inhibition of 17.3%, 4%, 21.7%, and 19.6%. A study conducted by Arslan Shah et al. (2024) showed that *Fagonia arabica* (FALE) leaves were used to synthesize gold nanoparticles, and it compared antibacterial tests and their zones of inhibition, which were done on *E. coli* and *P. multocida* and shown to be 12±0.1 and 18±0.5 (*Fagonia arabica* extract-stabilized gold nanoparticles as

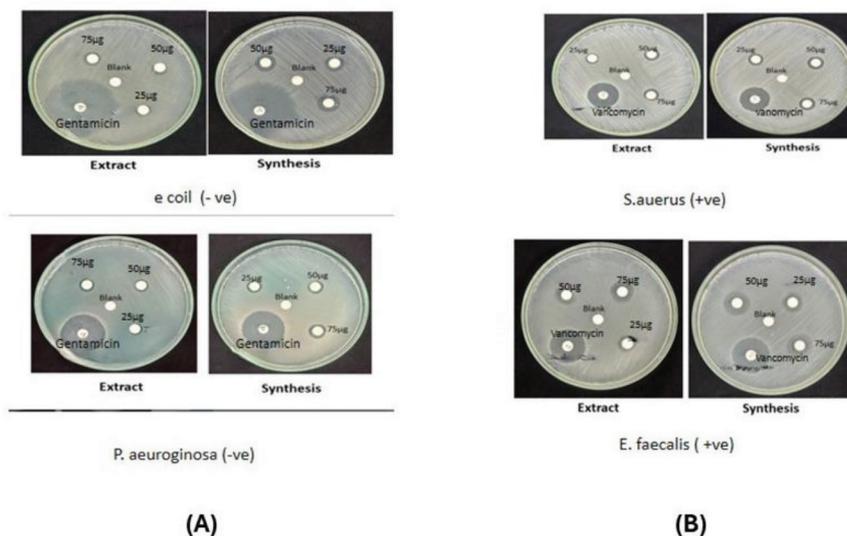


Figure 5 (A) Extract and synthesized CuNPs of *Dypsia lutescens* leaf extract with *E. coli* (-ve) and *P. aeruginosa* (-ve) bacteria and (B) Disc diffusion of bactericidal activity against the extract and synthesized CuNPs of *Dypsia lutescens* leaf extract with *S. aureus* (+ve) and *E. faecalis* (+ve) bacteria

a highly selective colorimetric nanoprobe for Cd²⁺ detection and as a potential photocatalytic and antibacterial agent). Hence, the use of metal-based nanoparticles has been shown to provide good antibacterial activity results with increasing dosages, and also indicates no toxicity.

Table 2 Zone of inhibition (mm) of *E. coli*, *P. aeruginosa*, *S. aureus*, *E. faecalis*, bacteria

Sl. No.	Sample name	Zone of inhibition (mm)			
		Bacteria <i>E. coli</i>	Bacteria <i>P. aeruginosa</i>	Bacteria <i>S. aureus</i>	Bacteria <i>E. faecalis</i>
A	Extract				
A.1	25 µg	3±0.22	4±0.31	5±1.22	7±1.13
A.2	50 µg	5±1.32	5±0.22	8±2.52	9±3.22
A.3	75 µg	6±0.43	8±0.52	10±4.22	11±2.11
	Gentamicin	39±3.22	27±1.32	NA	NA
	Blank	0	0	0	0
B	Synthesis				
B.1	25µg	13±1.53	9±0.22	10±2.22	12±0.33
B.2	50µg	15±4.22	10±0.31	11±1.42	16±0.52
B.3	75µg	16±2.42	12±0.12	13±1.22	18±0.34
	Gentamicin	39±2.07	27±0.33	NA	NA
	Blank	0	0	0	0

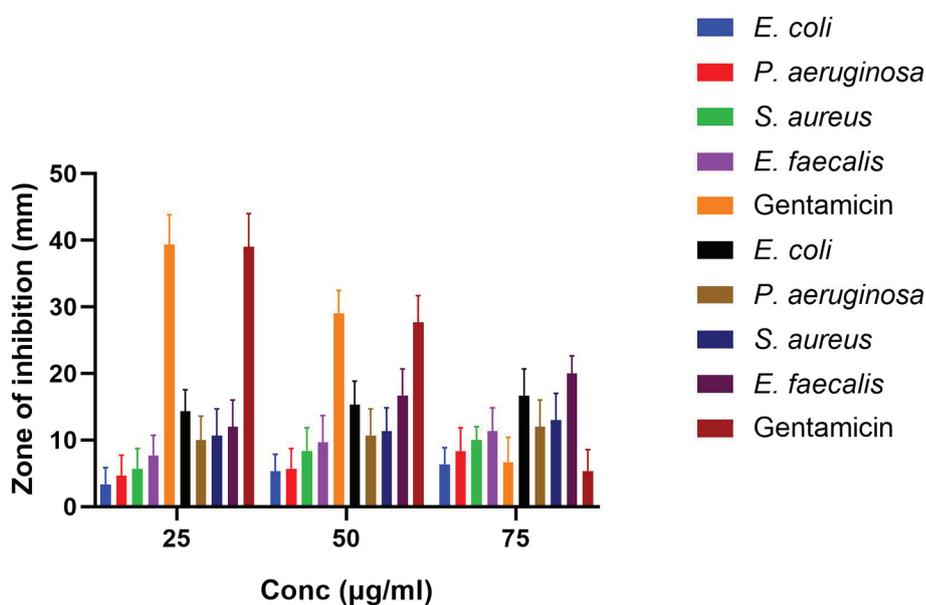


Figure 6 Effect of the zone of inhibition of copper nanoparticles against Gram-negative bacteria. Data was analyzed using two-way ANOVA with Tukey's post hoc test.

Discussion

In our study, copper nanoparticles were synthesized using a green synthesis approach employing *Dyopsis lutescens*. This plant has previously been reported to exhibit antibacterial, antimicrobial, and anti-inflammatory properties, according to our literature review. The nanoparticles were produced using an aqueous Soxhlet extraction method of the plant's leaves. As noted in earlier studies, copper acts as both a reducing and stabilizing agent. However, the green synthesis of copper nanoparticles utilizing *Dyopsis lutescens* leaves has not been documented before. This method yielded stable nanoparticles with significant bactericidal activity. Our findings underscore the potential of developing cost-effective copper nanoparticles for future biomedical applications.

Conclusion

Green synthesis of copper nanoparticles (CuNPs) using *Dyopsis lutescens* leaf extract was successfully achieved, demonstrating an eco-friendly and efficient approach. The presence of phytoconstituents, such as polyphenols, tannins, alkaloids, and flavonoids, played a significant role as reducing and capping agents, optimizing the synthesis process and enhancing the antibacterial properties of the CuNPs. UV-visible spectroscopy confirmed the formation of polyethylene glycol-coated CuNPs with a characteristic absorption peak at λ_{max} 410 nm. At the same time, FTIR analysis provided evidence of functional groups from the capping agents on the nanoparticle surface. The particle size analysis revealed a Polydispersity Index (Pdl) of 0.56, indicating acceptable size distribution, and the zeta potential of +40 mV confirmed the nanoparticles' excellent stability. The synthesized CuNPs exhibited significant antibacterial activity against Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and Gram-positive bacteria (*S. aureus* and *E. faecalis*), showing a slightly more extensive zone of inhibition compared to the plant extract alone. This study

highlights the synergistic potential of combining bioactive compounds from medicinal plants with CuNPs for practical antimicrobial applications, providing a sustainable solution for combating pathogenic microorganisms.

Author contributions

Abdul Rahamanulla: Writing the original draft, validation, visualization; Mohammed Gulzar Ahmed: Supervision; Ayesha Sultana: Formal analysis, review editing; Hanin Khadija: Data curation; Abdulla Ruknuddin: Data analysis.

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Conflict of interest

There are no potential conflicts of interest to declare.

References

1. Fokunang CN, Ndikum V, Tabi OY, Jiofack RB, Ngameni B, Guedje NM, et al. Traditional medicine: past, present and future research and development prospects and integration in the National Health System of Cameroon. *Afr J Tradit Complement Altern Med* 2011;8.
2. Painuli S, Semwal P, Cruz-Martins N, Bachheti RK. Medicinal plants of Himalayan forests. In: Husen A, Bachheti RK, Bachheti A, editors. *Non-Timber Forest Products: Food, Healthcare and Industrial Applications*. Cham: Springer; 2021;p.175-212.
3. Vert M, Doi Y, Hellwich KH, Hess M, Hodge P, Kubica P, et al.

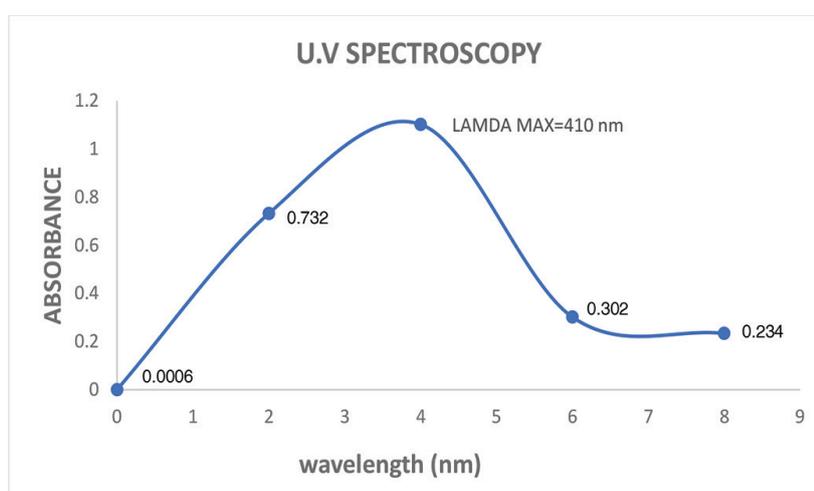
- Terminology for bio-related polymers and applications (IUPAC Recommendations, 2012). *Pure Appl Chem* 2012;84:377–410.
- Zahra Z, Habib Z, Chung S, Badshah MA. Exposure Route of TiO₂ NPs from industrial applications to wastewater treatment and their impacts on the agro-environment. *Nanomaterials* 2020;10:1469.
 - Ahmed SS, Sriramcharan P, Arivuselam R, Raman R, Venkatachalam S, Natarajan J. Green synthesis of bovine serum albumin-tailored silver nanoparticles from *Aspergillus fumigatus*: Statistical optimization, characterization, antioxidant, and cytotoxicity evaluation on colon cancer cells. *Appl Organomet Chem* 2024;38:e7386.
 - Mody VV, Siwale R, Singh A, Mody HR. Introduction to metallic nanoparticles. *J Pharm Bioallied Sci* 2010;2:282–9.
 - Schröfel A, Kratošová G, Šafařík I, Šafaříková M, Raška I, Šor LM. Applications of biosynthesized metallic nanoparticles—a review. *Acta Biomater* 2014;10:4023–42.
 - Kitchens CL, Hirt SM, Husson AA, Vertegel AS. Synthesis, stabilization, and characterization of metal nanoparticles [Dissertation]. Clemson (SC): Clemson University Graduate School; 2010.
 - Geoprincy G, Srri BV, Poonguzhali U, Gandhi NN, Ranganathan S. A review on green synthesis of silver nanoparticles. *Asian J Pharm Clin Res* 2013;6:8–12.
 - Tian K, Liu C, Yang H, Ren X. In situ synthesis of copper nanoparticles/polystyrene composite. *Colloids Surf A Physicochem Eng Asp* 2012;397:12–5.
 - Phong NTP, Khuong VQ, Tho TD, Van Du C, Minh NH. Green synthesis of copper nanoparticles colloidal solutions and used as pink disease treatment drug for rubber tree. *Proceedings of the 2011 International Workshop on Nanotechnology Applications (IWNA); 2011 Nov; Vietnam.* p. 10–2.
 - Saranyaadevi K, Subha V, Ravindran RE, Ranganathan S. Synthesis and characterization of copper nanoparticle using *Capparis zeylanica* leaf extract. *Int J Chem Tech Res* 2014;6:4533–1.
 - Sarpangala KB, Sarpangala M, Devasya A. Antimicrobial properties of Areca nut, Areca catechu, L: A review. *Int J Res Ayurveda Pharm* 2017;8:8–12.
 - Peng W, Liu YJ, Wu N, Sun T, He XY, Gao YX, Wu CJ. Areca catechu L.(Arecaceae): a review of its traditional uses, botany, phytochemistry, pharmacology and toxicology. *J Ethnopharmacol* 2015;164:340–56.
 - Jadhav S, Gaikwad S, Nimse M, Rajbhoj A. Copper oxide nanoparticles: synthesis, characterization and their antibacterial activity. *J Clust Sci* 2011;22:121–9.
 - Ejaz A, Mamtaz Z, Yasmin I, Shaban M, Siddique AB, Irfan MI, et al. *Cyperus scariosus* extract based green synthesized gold nanoparticles as colorimetric nanoprobe for Ni²⁺ detection and as antibacterial and photocatalytic agent. *J Mol Liq* 2024;393:123622.
 - Livermore DM. Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: our worst nightmare? *Clin Infect Dis* 2002;34:634–40.
 - Schwanninger M, Hinterstoisser B. Comparison of the classical wood extraction method using a Soxhlet apparatus with an advanced extraction method. *Eur J Wood Wood Prod* 2002;60:343–6.
 - Schwanninger M, Hinterstoisser B. Comparison of the classical wood extraction method using a Soxhlet apparatus with an advanced extraction method. *Eur J Wood Wood Prod* 2002;60:343–6.
 - PS SL, Ajith Babu TK, AyshathRizvana U, Mubashira KV, Mumthaz P. Phytochemical and pharmacological studies of flowers of areca catechu. *World J Pharm Res* 2020;9:1031–7.
 - Murthy HA, Desalegn T, Kassa M, Abebe B, Assefa T. Synthesis of green copper nanoparticles using medicinal plant *Hagenia abyssinica* (Brace) JF. Gmel. Leaf extract: antimicrobial properties. *J Nanomater* 2020;2020:3924081.
 - Kalainila P, Subha V, Ernest Ravindran RS, Sahadevan R. Synthesis and characterization of silver nanoparticle from *Erythrina indica*. *Asian J Pharm Clin Res* 2014;7:39–43.
 - Karthik C, Suresh S, Mirulalini S, Kavitha S. A FTIR approach of green synthesized silver nanoparticles by *Ocimum sanctum* and *Ocimum gratissimum* on mung bean seeds. *Inorg Nano-Met Chem* 2020;50:606–12.
 - Rodriguez-Loya J, Lerma M, Gardea-Torresdey JL. Dynamic light scattering and its application to control nanoparticle aggregation in colloidal systems: a review. *Micromachines (Basel)* 2023;15:24.
 - Zhang E, Zhao X, Hu J, Wang R, Fu S, Qin G. Antibacterial metals and alloys for potential biomedical implants. *Bioact Mater* 2021;6:2569–12.

Supplementary Table 1 Comparison of qualitative analysis of phytochemicals in both extracts

Phytochemicals	Methanol Extract	Aqueous Extract
Alkaloids	+++ (High)	+ (Low)
Flavonoids	+++ (High)	++ (Moderate)
Tannins	++ (Moderate)	+++ (High)
Saponins	+ (Low)	+++ (High)
Phenolics	+++ (High)	++ (Moderate)
Carbohydrates	+ (Low)	+++ (High)

Supplementary Table 2 Analysis of UV spectroscopy

	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>E. faecalis</i>
<i>Dypsis lutescens</i>	12.3	4.21	12.42	10.44

**Supplementary Figure 1** UV spectroscopy of CuNPs from *Dypsis lutescens* leaf extract