Copper nanoparticles synthesis from *Andrographis paniculata* leaf extract– characterization and its application

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Copper nanoparticle synthesis and characterization are now being done widely due to its broad nanotechnology research interest, especially in medical applications. The current work set out to produce copper nanoparticles(CuNPs) by employing the herb *Andrographis paniculata* for medicinal purposes. *Andrographis paniculata* leaf extract was used to make CuNPs using copper sulphate ($CuSO_4$). For monitoring the synthesis of CuNPs, the UV-vis absorption spectra were obtained and Surface Plasmon resonance (SPR) peaks at 500nm. The X-ray diffraction (XRD) pattern reveals the average size of the crystallites is 5.876 nm. This environmentally friendly approach yields homogenous, spherical particles, as demonstrated by images of Scanning and Transmission electron microscopy (TEM and SEM). The outcomes demonstrated that leaf extract is most suited for producing CuNPs. It shows a greater zone of inhibition and inhibitory action against tested bacteria such as *Bacillus cereus, Escherichia Coli, Klebsiella pneumoniae* and *Staphylococcus aureus,* and it can be used for the inhibition of several harmful microbes. Because the synthesized CuNPs are non-toxic, environmentally benign, and suitable for usage in pharmaceutical and other fields, they can be applied in a variety of ways in future.

(Received May 16, 2024; Accepted September 9, 2024)

Keywords: *Andrographis paniculata*, Phytochemical study, Antimicrobial activity, Copper nanoparticles, Characterization

1. Introduction

Modern research is greatly influenced by nanotechnology, which is the most versatile technology available and can be used in nearly all industries, including biomedical science, gene delivery, healthcare, electronics, pharmaceutical food & feed, energy research, cosmetics, environmental health mechanics, and space industries. Much interest has been shown in CuNPs because of their superior chemical and physical characteristics. A wide range of devices, including beat transmission systems, catalysts, extremely strong materials, and antibacterial materials, can be made with CuNPs since a strong surface-to-volume ratio and strong reactivity characterize CuNPs and they can readily combine with other particles to intensify their antibacterial properties [1]. Similar to other types of nanoparticles, CuNPs can be created chemically or naturally with a size range from 1 to 100 nm. Copper is a good alternative for Nobel metals such as gold, and silver as it is highly conductive and economical gained much importance due to its low-cost preparation and has excellent physical cum chemical properties copper oxide nanoparticles possess a surface-to-volume ratio that makes them highly reactive, and they also interact along other particles with ease.

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https://doi.org/10.15251/JOBM.2024.163.151

Because this process may be easily scaled up, material-based plants are typically cited as the source of explanation for the synthesis of CuNPs [2, 3].

Andrographis paniculata belongs to the family *Acanthaceae*, is one of the most commonly used medicinal plants in the world. Throughout history, it has been utilized to address numerous ailments such as cancer, diabetes, elevated blood pressure, ulcers, itchiness, bronchitis, skin disorders, gas, flu, diarrhea, dyspepsia, and malaria [4]. It has several photochemical components with pharmacological effects that are both elegant and captivating. To fill up available research capacity, this work denotes the current study being done on *Andrographis paniculata* about pharmacological usage, photochemistry, pharmacological activity, and therapeutic application. Their tight interaction with bacteria membranes and the metal ions they release into solutions cause antimicrobial activity. Next, the lipids in a cell membrane are disassembled by free radicals via oxidation, causing the membrane to degenerate [5]. Using leaf extracts from *Andrographis paniculata*, CuNPs were synthesised and characterised in the current study. Additional copper nanoparticle characterization included antibacterial activity, structural characterisation using X-ray diffraction (X-RD), optical characterization with a UV-VIS spectrometer, and scanning electron microscopy (SEM).

2. Experimental

2.1. Collection of fresh leaves

The local area in and around Erode, Tamil Nadu, was used to gather the fresh leaves of *Andrographis paniculata.*

2.2. Preparation of aqueous plant extracts

After gathering the *Andrographis paniculata* leaves, all impurities were removed by first cleaning them with ordinary tap water and then with distilled water. It chopped the leaves into little pieces. The combination was boiled for 15 minutes at 60 degrees in a water bath after 10g of leaves and 100 ml of distilled water were combined. To store the extract for future use, it was maintained at room temperature in a beaker that was tightly sealed with aluminium foil.

2.3. Phytochemical screening

The phytochemical screening was performed according to the methods of [6]. The following substances were tested for analysis using the *Andrographis paniculata* aqueous extract.

2.4. Preparation of copper sulphate solution

The solution used in the synthesis of CuNPs was analytical-grade copper sulphate. 2mM stock copper sulphate in chloride-free distilled water was prepared for 100ml using Copper sulphate (0.2496 g) in a 250 ml beaker. The beaker holding the solution was kept out of direct sunlight and tightly covered with aluminium foil to preserve it for use at a later time[7].

2.5. Synthesis of CuNPs

After pipetting out 80 ml of $CuSO_4$ solution into a dry, clean 250 ml beaker and setting it on a magnetic stirrer, 20 ml of prepared *Andrographis paniculata* leaf extract was gradually added to create the CuNPs.The solution's colour turns from pale green to dark green when the extract is added. The solution was tightly covered and allowed for 24 hours at room temperature. After 24 hours in a hot air oven at 300 degrees C for 24 hours, the solution was dried and collected the dry powder for further use.

2.6. Characterisation of synthesized CuNPs

2.6.1. UV – analysing spectroscopy

With absorbance at 200-600 nm and a peak maximum of nm, the typical Surface Plasmon Resonance (SPR) spectra from the nanoparticle solution's UV–visible spectroscopy were revealed. This is because CuNPs production is suspected of producing these spectra. The initial colour change

indicated that the production of the copper nanoparticles was successful. The size and form of regulated nanoparticles in aqueous suspension could be investigated using it. About 24 hours separated the times when the UV-Vis spectra were recorded [8].

2.6.2. Fourier transform infrared (FT-IR) spectroscopy

Finding the molecules and their functional groups in the samples is done using FTIR characterisation. The dried copper-based nanoparticles were subjected to FTIR analysis for analysis of the capping ligand of CuNPs which act as a stabilizing agent [9].

2.6.3. Scanning electron microscopy (SEM) – investigation

To determine the surface morphology of the nanoparticles, a scanning electron microscopy investigation was performed. The analysis focuses on the hydrogen bonds and electrostatic interactions among the bio-organic capping molecules that are in charge of producing CuNPs through the use of plant extract.

2.6.4. X-ray diffractometer (XRD) - analysis

Using an X-ray diffractometer, the heat-dried nanoparticles were examined to determine whether CuNPs had formed. Scherer's formula [10] was utilized to determine the grain size based on the diffracted intensities, which were measured between 10 and 90 degrees of 2θ angles.

2.6.5. Antibacterial characteristics of synthesized CuNPs

Klebsiella pneumonia, *Bacillus cereus*, *Escherichia coli*, and *Staphylococcus aureus* were among the pathogens against which the microbiological activity of CuNPs synthesized using *Andrographis paniculata* aqueous leaf extract was assessed. The agar well diffusion method was used to test the antibacterial activity. Human infections were used in the antibacterial test for the synthesized CuNPs. These test cultures were swabbed on the solidified Muller Hinton Agar using sterile cotton swabs. The wells were created by agar well puncture. The CuNPs sample was added to the well at different concentrations (50 - 100mμ) and control was observed and recorded in the diameter of the inhibition zone as millimetres (mm) [11].

3. Results and discussion

3.1. Phytochemical analysis of *andrographis paniculata* plant leaf extract

The phytochemical analysis of *A. Paniculata* leaf extract showed the presence of some compounds in the plant.

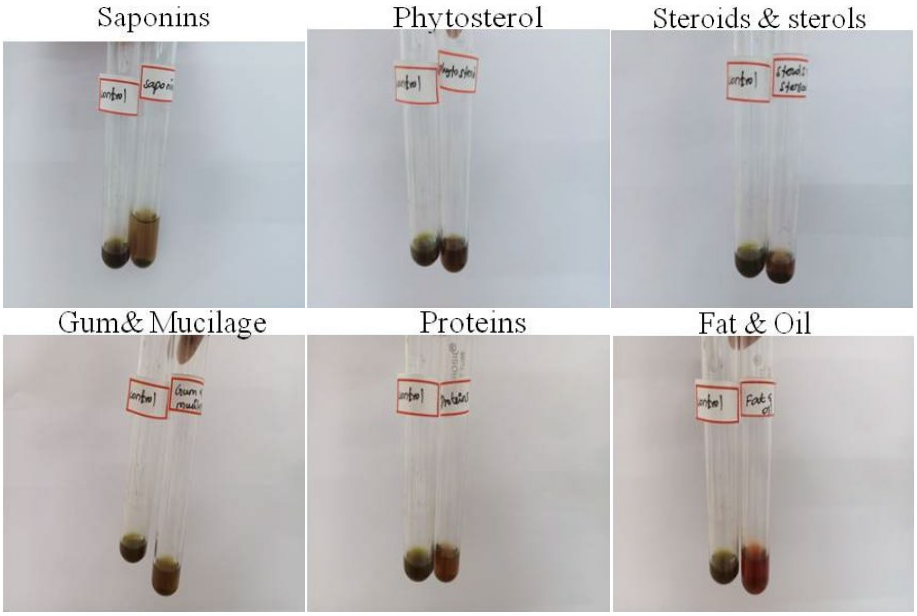

Fig. 1. Phytochemical analysis of a. paniculata plant leaf extract.

In this extract, fifteen compounds were tested, and six were present. The phytochemical examination of *A. paniculata* aqueous leaf extract revealed the presence of sterols and steroids, saponins, Phytosterols, proteins, fat and oil, gum and mucilage. The result is shown in Figure 1 and is listed in Table 1.

Table 1. Phytochemical analysis of A. paniculata.

S.No	COMPONENTS	RESULTS
1	Alkaloids	Absent
2	Tannins	Absent
3	Flavanoids	Absent
4	Steroids & Sterols	Present
5	Terpenoids	Absent
6	Phenols	Absent
7	Saponins	Present
8	Pytosterols	Present
9	Glycosides	Absent
10	Flavanoids	Absent
11	Carbohydrates	Absent
12	Proteins	Present
13	Amino acids	Absent
14	Fat & Oil	Present
15	Gum & Mucilage	Present

3.2. Copper nanoparticle synthesis

CuNPs production in the solution of 2 mM copper sulphate and aqueous extract of the *A. paniculata* plant sample was confirmed by a change in the colour from light green to dark green and was shown in Figure 2 and the purified CuNPs were shown in Figure 3.

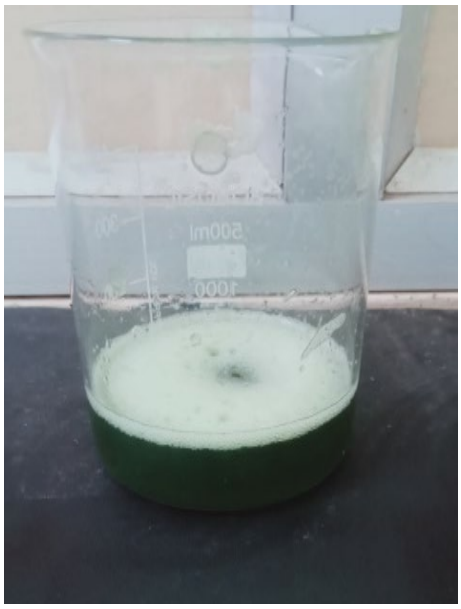

Fig. 2. Green colour formation of CuNPs using A. paniculataleaf extract and copper sulphate.

Fig. 3. Purified CuNPs.

3.3. Characterisation of Cu NPs

3.3.1. UV-visible spectroscopy

Generally, metallic elements Cu NPs were celebrated to exhibit SPR development wherever conducting electrons in metal oscillate together in resonance with bound wave length upon interaction with an associate degree magnetic force field. These SPR bands highly depend on the type, size, and shape of the Cu NPs and the surrounding environment. UV-visible spectroscopy displays an intense SPR peak at 420 nm for CuNPs (Figure: 3.6). The synthesised Cu was stable at room temperature even after 45 days [12].

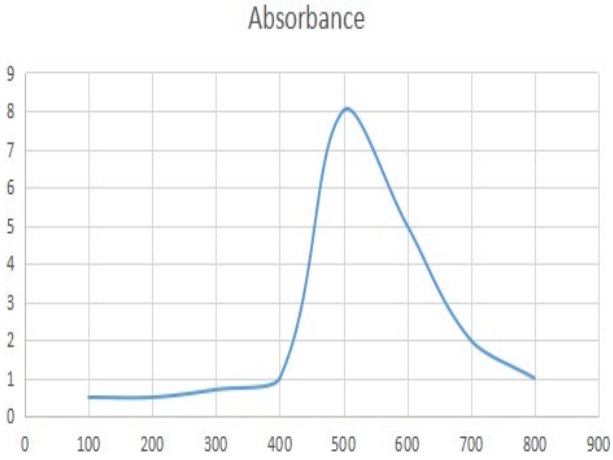

Fig. 4. UV visible spectrum of synthesized Cu NPs.

3.3.2. Fourier transform infrared spectroscopy (FTIR) analysis

FTIR interpretation of CuNPs had shown the peak at 601.79cm-1, 648.08 cm-1,802.39cm-1, 1072.42 cm-1, indicating the presence of stretch corresponds which indicated the presence of C-H Stretch which corresponds to Alkynes, N-H Stretch which corresponds to 1° and 2° amine, C-O Stretch which corresponds to alcohols, carbohydrates, esters, ethers respectively (Figure 5).

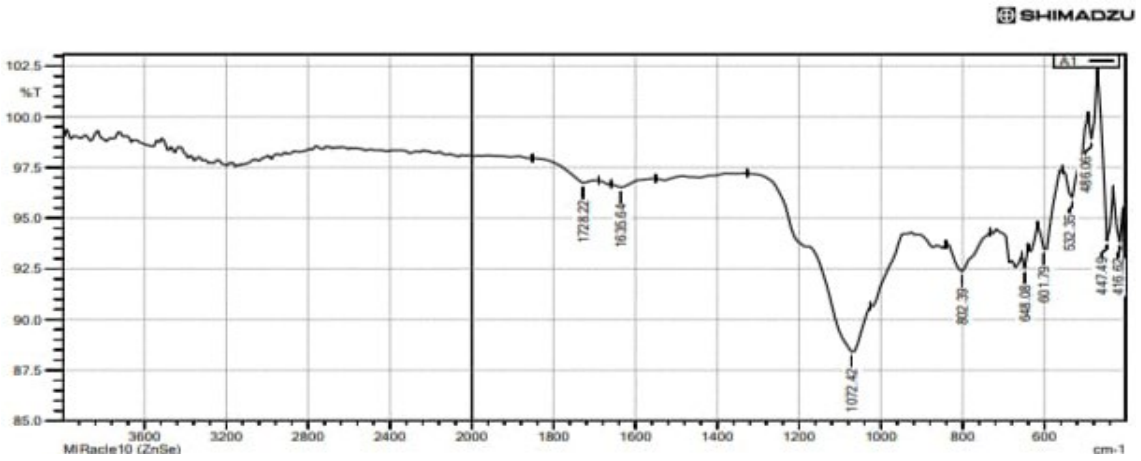

Fig. 5. FTIR Analysis of synthesized CuNPs.

3.3.3. XRD analysis

The XRD analysis was observed with 2θ values as 18.2906°,25.6554°,31.0181°,36.3967°, 43.6845°,44.8468°, 52.6266°, 56.3962° corresponding to the crystalline structure of copper (Figure 6) [13, 14].

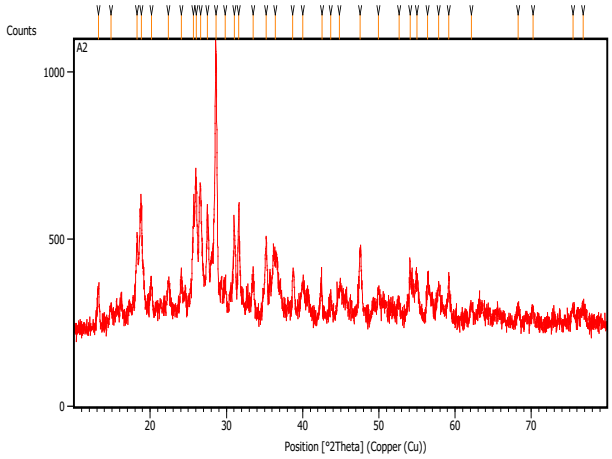

Fig. 6. XRD analysis shows the crystalline Cu NPs.

3.3.4. SEM analysis

The size and morphology of nanoparticles were characterized. SEM micrograph shows the fine configuration of spherical Cu NPs with size ranges between 10-35 nm (Figure 7) with an average size of 15±9. It was also found that the Cu NPs had a thin layer of biomolecule on its surface; particles were polydispersity and stable for a long period. In the previous literature, considerable studies have been reported [15, 16].

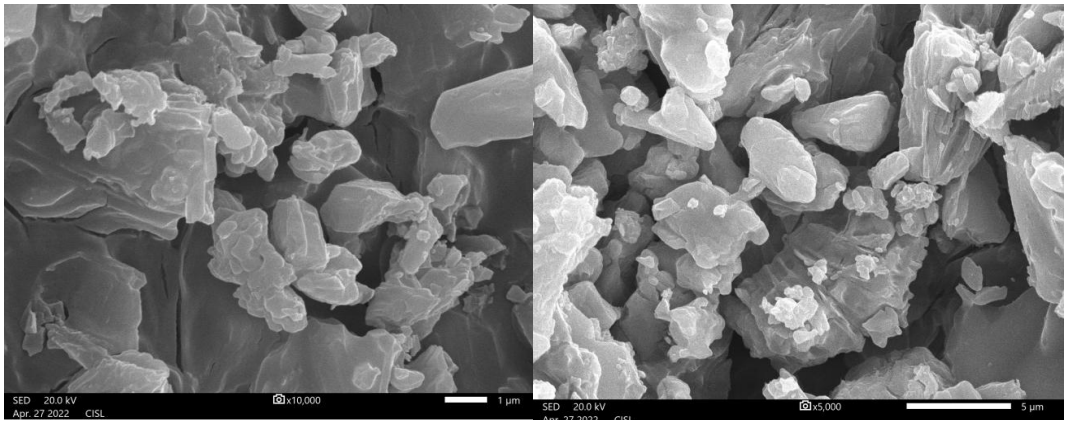

Fig. 7. SEM micrographsofsynthesized CuNPs display well-dispersedspherical shape particles with a size range between 10-50 nm.

3.4. Antimicrobial activity of copper nanoparticles

The zone of inhibition in the plate showed the CuNPs synthesised using an aqueous extract of *A. Paniculata* had antimicrobial activity against bacterial test pathogens namely *E. coli, B. cereus, S. aureus* and *K.pneumoniae*. The zone of inhibition was found to be higher against *Bacillus cereus and* was shown in (Figure 8) and the diameter values were given in Table 2. [17-22].

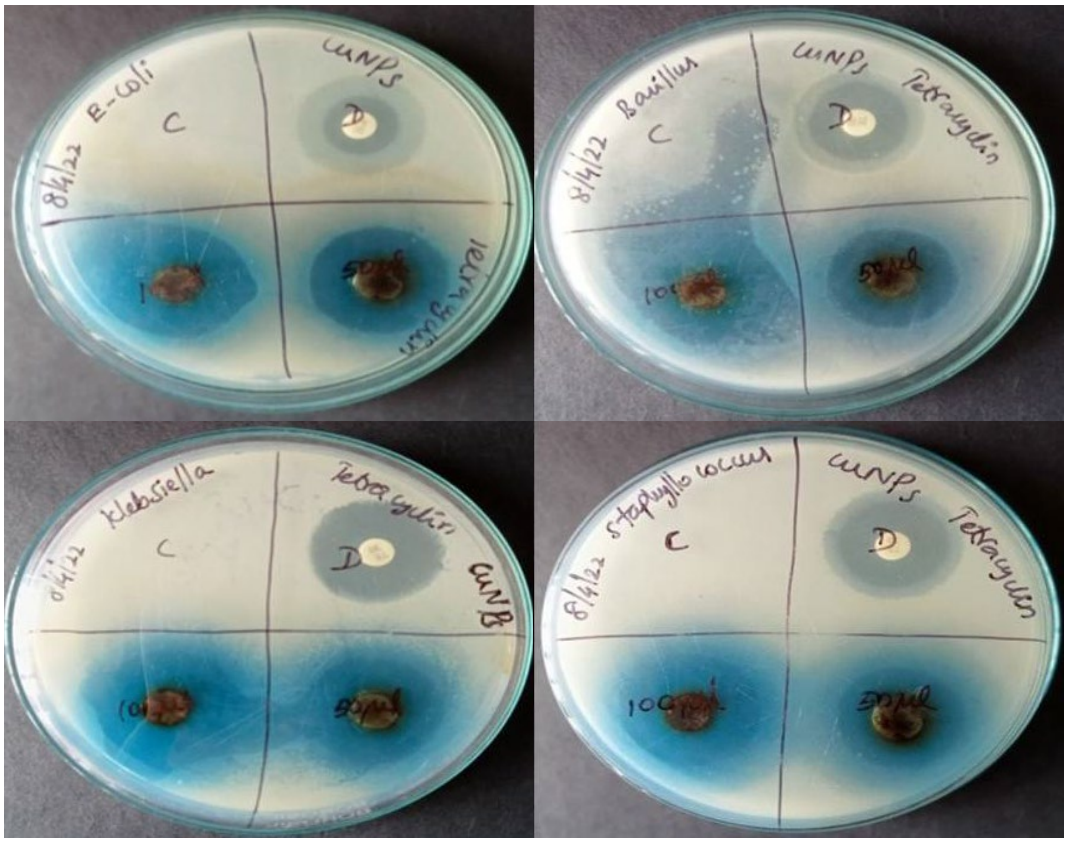

Fig. 8. Antimicrobial Activity of CuNPsfrom Andrographis paniculata.

Table 2. Antimicrobial Activity of Copper Nanoparticles.

Test Microorganism	Zone of Inhibition (mm)		
	Copper nanoparticles 50 µl	Copper nanoparticles 100 µl	Tetracycline 25 (mcg)
Staphylococcus aureus(Gram +ve) (MTCC No.: 3160)	17	20	13
Bacillus cereus(Gram +ve) (MTCC No.: 0430)	24	25	13
Escherichia coli(Gram -ve) (MTCC No.: 1698)	23	25	13
Klebsiellapneumoniae(Gram -ve) (MTCC No.: 1030)	15	18	13

3.5. Antioxidant activity

In the present investigation, the antioxidant activity of aqueous extract of *A.paniculata* and CuNPs was studied by using the DPPH method. The present study showed the aqueous extract *A. paniculata* has potential antioxidant activity. It results are shown in the graph (Figure 9). Concentration and scavenging activity values are given in Table 3.

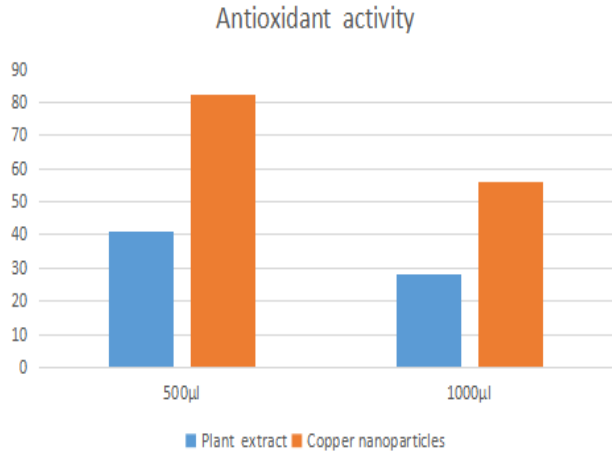

Fig. 9. Antioxidant activity by DPPH method.

Table 3. Antioxidant activity by DPPH method.

S.No	Concentration of sample (µl)	Scavenging Activity (%)	
		Plant extract	CuNPs
1	500	41	28
2	1000	82	56

5. Conclusion

The Indian traditional medical system is based primarily on medicinal herbs. Research on pharmacology has recognized the importance of therapeutic plants as a possible source of bioactive substances.To sum up, the creation of dependable and environmentally benign methods for the manufacture of metal nanoparticles is the focus of the discipline of nanotechnology. For their effective antimicrobial properties, we have reported a straightforward biological and inexpensive method for creating stable CuNPs by reducing copper sulphate solution using a bio-reduction technique and an *Andrographis paniculata* aqueous extract of the entire plant as the reducing agent. XRD and SEM techniques were used to examine the morphological and structural properties of the

produced CuNPs. The outcome validated the conversion of copper sulphate into very stable, impurity-free CuNPs. The UV-Vis analysis was used to investigate the optical properties of CuNPs and has been confirmed by the peak in the absorption spectra. The disc diffusion method was used to study the additional antibacterial activity of plant extract and artificial CuNPs. According to the findings, other harmful microbes, including *E.coli, B. cereus, S. aureus* and *K. pneumoniae* can also be inhibited from growing by the copper nanoparticles derived from the extract of the *A. paniculata* plant.

Acknowledgements

The authors are grateful to the Researchers Supporting Project number (RSP2024R360), King Saud University, Riyadh, Saudi Arabia and the authors also thanks to the PG & Research of Biotechnology of Nandha Arts and Science College, Erode, Tamil Nadu, India for providing the necessary research facilities to conduct this study.

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