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Research Article

Green Synthesis of Copper Nanoparticles with Adhatoda Vasica: Antibacterial and Antioxidant Study

Faruk Arodiya*[1], Nahid Malik[1], Chirag Makvana[2], Kokila Parmar[1]

[1]Department of Chemistry, Hemchandracharya North Gujarat University, Patan-384265, India.
[2]Department of Chemistry, Gokul Global University, Sidhpur-384151, India.
*Correspondence: Faruk Arodiya (farukarodiyachem4@gmail.com)

Abstract

The burgeoning field of green nanotechnology has spurred the interest of researchers towards environmentally responsible nanoparticle production. In this study, Aradusi leaf extract was utilized for the synthesis of stable copper nanoparticles, subsequently functionalized with Polyvinyl Pyrrolidone (PVP) polymer. A comprehensive characterization of these biosynthesized nanoparticles was conducted using UV–Visible spectrophotometry, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and transmission electron microscopy (TEM). The UV–visible absorption spectra of bio-reduced copper nanoparticles were analyzed to assess their stability, while their antibacterial activity was evaluated against both gram-negative and gram-positive microbes. Additionally, their antioxidant potential was determined through DPPH free radical scavenging assays. Aradusi leaf extract demonstrated proficient reduction of copper ions into copper nanoparticles. Consequently, this methodology offers a rapid and environmentally benign route for the synthesis of stable copper nanoparticles exhibiting antibacterial and antioxidant activities within the size range of 10-100 nm, showcasing their potential applications in medical science.

Keywords: Antibacterial, antioxidant, nanoparticles, aradusi, polymer functionalized, green synthesis

1. Introduction

Nanostructures, characterized by their diverse physical, chemical, and electrical properties, have found widespread applications across various fields including antimicrobial treatments, optics, electronics, catalysis, energy conversion, storage devices, and biotechnology [1-4]. Among the plethora of materials available, copper and its alloys have garnered significant attention due to their versatile properties, finding applications in electrical engineering, catalysis, optics, and as potent antibacterial and antifungal agents [5]. The unique properties of copper nanoparticles position them as promising alternatives to noble metals such as gold, palladium, silver, and platinum, with applications spanning biosciences, biomedicine, catalysis, dielectrics, imaging, magnetism, and beyond [6].

In the quest for sustainable and environmentally benign nanoparticle synthesis methods, the utilization of medicinal plants has emerged as a cost-effective, abundantly available, and non-toxic approach suitable for industrial-scale production. In recent years, a variety of biological entities including algae [7], bacteria [8], fungi [9], mushrooms [10], enzymes [11], and plant leaf extracts [12] have been harnessed for the fabrication of metallic nanoparticles, offering advantages such as non-toxicity, energy efficiency, cost-effectiveness, and eco-friendliness. Plants, in particular, offer a favorable platform for nanoparticle synthesis as they inherently lack hazardous chemicals and possess natural capping agents, thereby eliminating the need for synthetic stabilizers. Furthermore, the use of plant extracts reduces the costs associated with microbial isolation and culture media, enhancing the cost-competitive viability of microorganism-based nanoparticle synthesis. In this study, we focus on the rapid synthesis of copper nanoparticles utilizing

extract from Ocimum sanctum leaves. Ocimum sanctum, commonly known as Tulsi, is a traditional Indian medicinal plant renowned for its potent bio-reduction and stabilization properties. Tulsi leaves contain a rich array of bioactive compounds including alkaloids, glycosides, tannins, saponins, aromatic compounds, and essential minerals such as calcium, manganese, copper, zinc, phosphorus, potassium, sodium, and magnesium, with copper content notably higher compared to other leaf sources, standing at 12.31 mg/kg [13]. Among its constituents, urosolic acid emerges as a primary active ingredient, contributing to Tulsi's therapeutic properties and its efficacy as a reducer.

The aqueous chemistry of Tulsi extract, powered by compounds like gallic acid, has been instrumental in reducing silver ions to silver nanoparticles, highlighting its potential as a versatile reducing agent [14]. Recent studies have also demonstrated the efficacy of Ocimum sanctum leaf extracts in the synthesis of silver and gold nanoparticles, leveraging its inherent bio-reducing and stabilizing capabilities [15]. Given copper's well-established antimicrobial properties, various plant extracts including Citrus Lemon fruit, Green coffee bean, Neem flower, Citrus paradisi fruit peel, Hibiscus rosa sinensis, Ocimum sanctum, Syzygium aromaticum (Cloves), Vitis vinifera, Eucalyptus, Cassia alata, Centella asiatica, Malva sylvestris, and others, have been employed for the synthesis of copper nanoparticles [20]. Notably, capsicum frutescens leaf extract has also been explored for this purpose [21].

2. Materials and methods

2.1. Preparation of leaf extract

Fresh Aradusi leaves (5 g) were thoroughly washed with distilled water twice and dried on filter paper to remove residual moisture. Subsequently, the leaves were placed in a clean beaker and 100 mL of distilled water was added using a measuring cylinder. The mixture was heated to obtain the leaf extract, which was then stored in an amber-colored bottle in a refrigerator.

2.2. Synthesis of copper nanoparticles

A 25 mL portion of the Aradusi leaf extract was mixed with 100 mL of a 1 mM aqueous solution of copper sulphate pentahydrate ($CuSO_4\ 5H_2O$) under continuous stirring. After complete mixing of the leaf extract with the precursor, the mixture was incubated at 31°C for 24 hours. The formation of copper nanoparticles was indicated by a color change from pale green to light yellowish. Subsequently, the solution was centrifuged at 6000 rpm for 30 minutes, and the pellet obtained was re-dispersed in deionized water to remove any unwanted biological contaminants.

2.3. Synthesis of polymer functionalized copper nanoparticles

In 100 mL of ultra-pure water, 0.2 g of Polyvinyl Pyrrolidone (PVP) was dissolved and stirred for 1 hour at 80°C. The resulting solution was gradually added to the homogeneous solution of copper nanoparticles obtained from the leaf extract. After 1 hour, the light yellowish color of the mixture turned into a dark yellow hue (Figure 1). The reaction mixture was allowed to cool for 10 minutes before being centrifuged at 10000 rpm for 15 minutes. The precipitates formed were washed with deionized water and then dried in a 70°C oven for 24 hours.

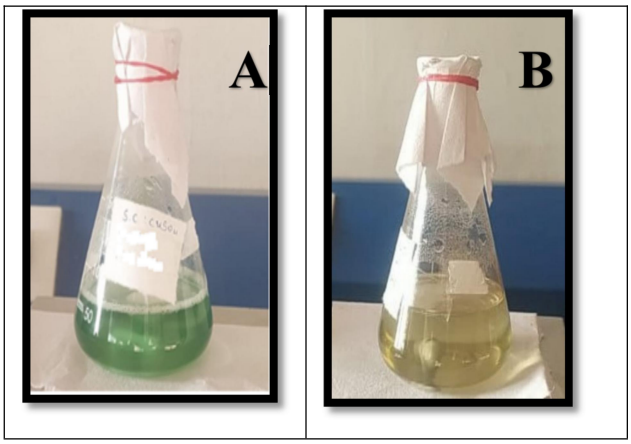

Figure 1. Colour Change from light green to yellowish [A] After 24 hours [B] After adding PVP solution

2.4. Characterization of green CuNPs and PVP functionalized CuNPs

Characterization of the green CuNPs and PVP-functionalized CuNPs involved employing various contemporary techniques. The production of CuNPs and polymer-functionalized CuNPs

was verified using a UV-visible spectrophotometer (Perkin Elmer USA). Additionally, FTIR analysis spanning the 500 - 4000 cm^-1 range was conducted to confirm the presence of functional biomolecules associated with both types of nanoparticles. To ensure purity, XRD technique was utilized with a Rigaku D/max 40 kV X-ray diffraction spectrometer. Furthermore, the structural morphology of the synthesized nanoparticles was analyzed using high-resolution transmission electron microscopy (HR-TEM).

2.5. Anti-microbial activity

The antibacterial activity of the synthesized CuNPs was assessed using a modified version of the well diffusion method outlined by Hulikere et al. [22]. Overnight cultures of all test bacterial strains were grown in nutrient broth at 37°C and adjusted to a McFarland standard of 0.5. Under sterile conditions, 100 µL of each Gram-positive strain (Bacillus subtilis and Staphylococcus aureus) and each Gram-negative strain (Pseudomonas aeruginosa and Escherichia coli) were spread onto individual nutrient agar plates. Using a cork borer, wells with a diameter of 10 mm were punched into the agar plates, and the synthesized CuNPs and PVP-functionalized CuNPs were inoculated into each well. Additionally, 100 µL of streptomycin (1 mg/mL) served as a positive control. The plates were then incubated at 37°C for 24 hours, after which the antibacterial activity was assessed by measuring the diameter of the inhibition zone using a zone scale (HiMedia).

2.6. Antioxidant activity

The antioxidant properties of the synthesized CuNPs and PVP-functionalized CuNPs were evaluated using the DPPH method [23], with ascorbic acid chosen as the standard due to its high antioxidant activity. Standard solutions of ascorbic acid, as well as various concentrations (10, 20, 30, 40, 50, 75, 100 µg/mL), were prepared. DPPH was prepared by dissolving 20 mg of the compound in 100 mL of methanol. Subsequently, 1 mL of the various concentrations of CuNPs, PVP-functionalized CuNPs, and the standard ascorbic acid solution were separately mixed with 1 mL of the DPPH solution and incubated for 30 minutes. The absorbance was then measured using a UV-Visible Spectrophotometer at 517 nm. The free radical scavenging activity was expressed as the percentage of inhibition, calculated using the following formula.

$$\%\ \text{of Antioxidant activity} = \left(\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absobance of control}}\right) \times 100$$

3. Results and discussion

3.1. UV–Visible Spectroscopic Analysis

UV–visible spectroscopy confirmed the formation of copper nanoparticles (CuNPs) via aqueous-phase reduction (Figure 2). A distinct color change from light yellow to dark yellow was observed, attributed to the excitation of surface plasmon resonance (SPR), confirming nanoparticle synthesis. Absorbance peaks for CuNPs and PVP-functionalized CuNPs were observed at 322 nm and 247 nm, respectively. These variations reflect the influence of nanoparticle size and morphology on SPR behavior, consistent with previous studies [24–28].

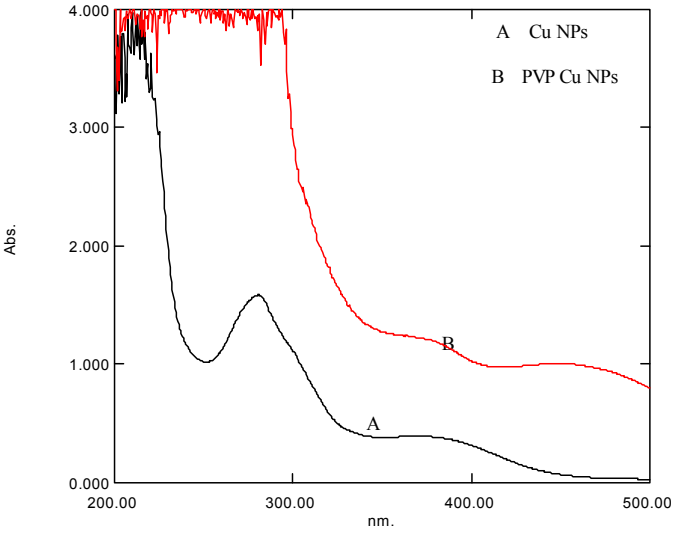

Figure 2. UV Visible spectrum of [A] CuNPs [B] PVP CuNPs.

3.2. FTIR Analysis

FTIR spectroscopy was used to identify functional groups involved in the bioreduction and stabilization processes (Figure 3).

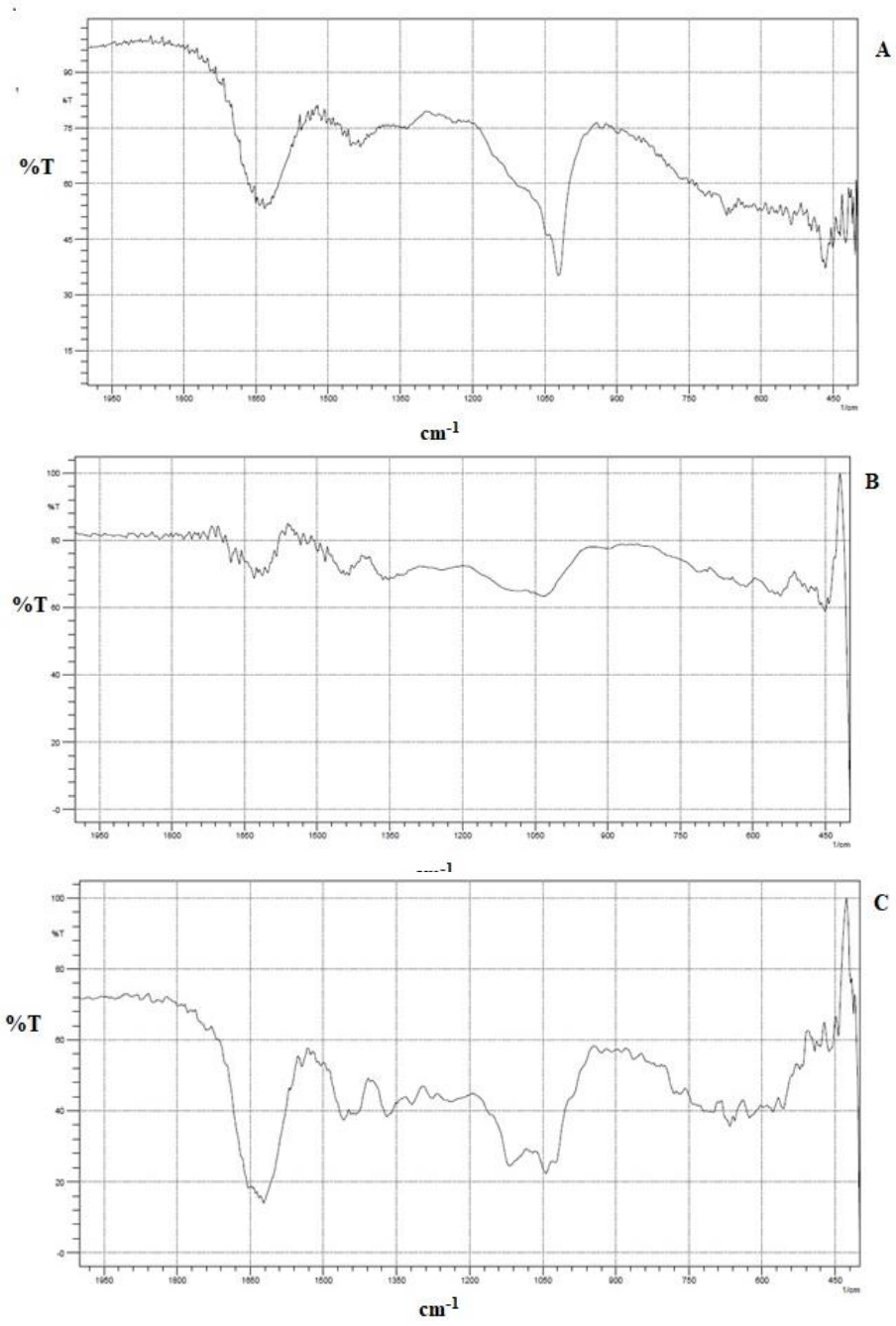

Figure 3. FT-IR Spectrum of [A] Aradusi leaf extract [B] CuNPs [C] PVP CuNPs.

Characteristic absorption bands appeared at 1653 cm^{-1} (C=C stretching), 1100 and 1700 cm^{-1} (C–O and C=O stretching), 610 cm^{-1} (indicative of CuNP formation), 1480–1320 cm^{-1} (C–H bending), and 1024 cm^{-1} (C–X stretching). These results suggest the presence of bioactive molecules, such as amino acids and phenolic compounds, that act as natural capping agents, enhancing nanoparticle stability and preventing aggregation [29–31].

3.3. HR-TEM analysis

HR-TEM imaging revealed uniformly distributed, spherical PVP-CuNPs with an average particle size of 73.50 nm. The selected area electron diffraction (SAED) pattern exhibited

well-defined circular spots, further confirming the crystalline nature of the synthesized nanoparticles (Figure 4, 5 & 6).

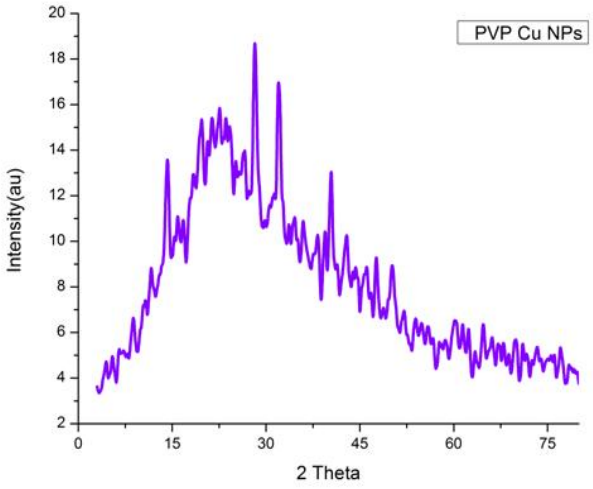

Figure 4. XRD pattern of PVP functionalized CuNPs.

3.4. Antibacterial activity

The synthesized CuNPs and PVP-functionalized CuNPs exhibited significant antibacterial activity against both Gram-positive and Gram-negative bacteria (Figure 7). PVP-CuNPs showed superior inhibition zones compared to CuNPs and even surpassed streptomycin in some cases. The enhanced efficacy of PVP-CuNPs may be attributed to the improved dispersion, bioavailability, and surface reactivity conferred by polymer functionalization.

The data clearly indicate that PVP-functionalized CuNPs exhibited the highest antibacterial activity against all tested organisms, with inhibition zones ranging from 18 to 22 mm. This suggests that the polymeric capping significantly enhances the antimicrobial efficacy of CuNPs.

Among the bacterial strains, Pseudomonas aeruginosa showed the greatest susceptibility to PVP-CuNPs (22 mm), comparable to the standard antibiotic streptomycin (23 mm), followed by Escherichia coli (22 mm) and Bacillus subtilis (19 mm). In contrast, the $CuSO_4$ solution and plant extract exhibited relatively lower antibacterial effects, with inhibition zones not exceeding 18 mm.

The enhanced antibacterial activity of PVP-CuNPs can be attributed to several synergistic factors:

- Improved dispersion and stability of the nanoparticles in aqueous media due to PVP coating, which increases surface availability and interaction with bacterial cells.
- Sustained release of copper ions (Cu^{2+}) from the nanoparticle core, prolonging their bactericidal action.

Electrostatic and hydrogen-bonding interactions between PVP functional groups and bacterial membranes, which may facilitate enhanced uptake or membrane disruption.

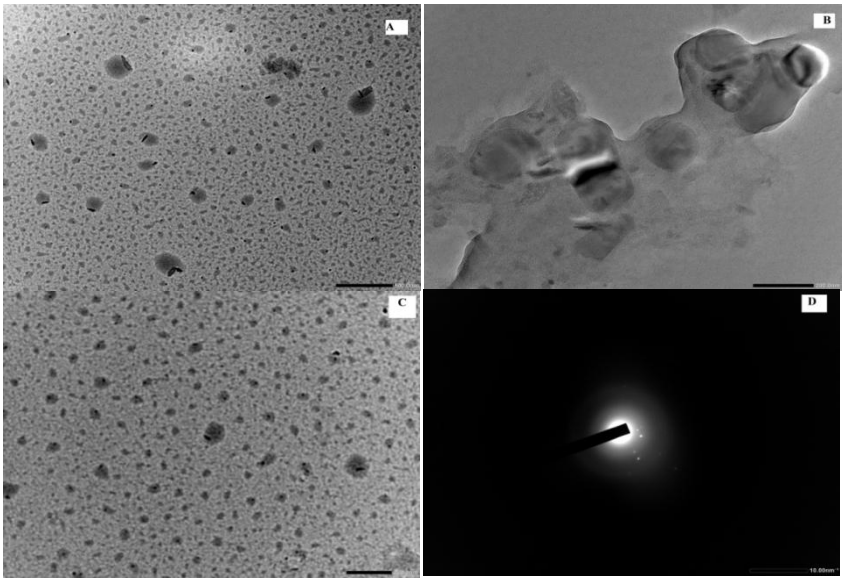

Figure 5. HR-TEM image [A], [B], [C] and SAED image [D] of PVP CuNPs.

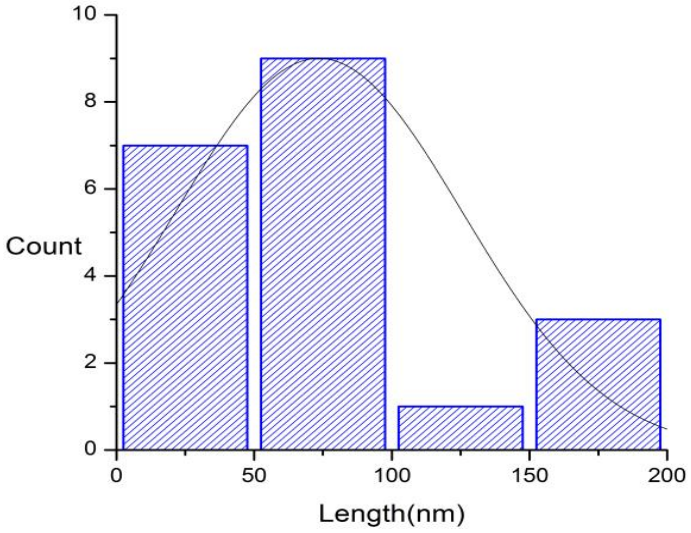

Figure 6. The size distribution curve from the TEM analysis of PVP functionalized CuNPs.

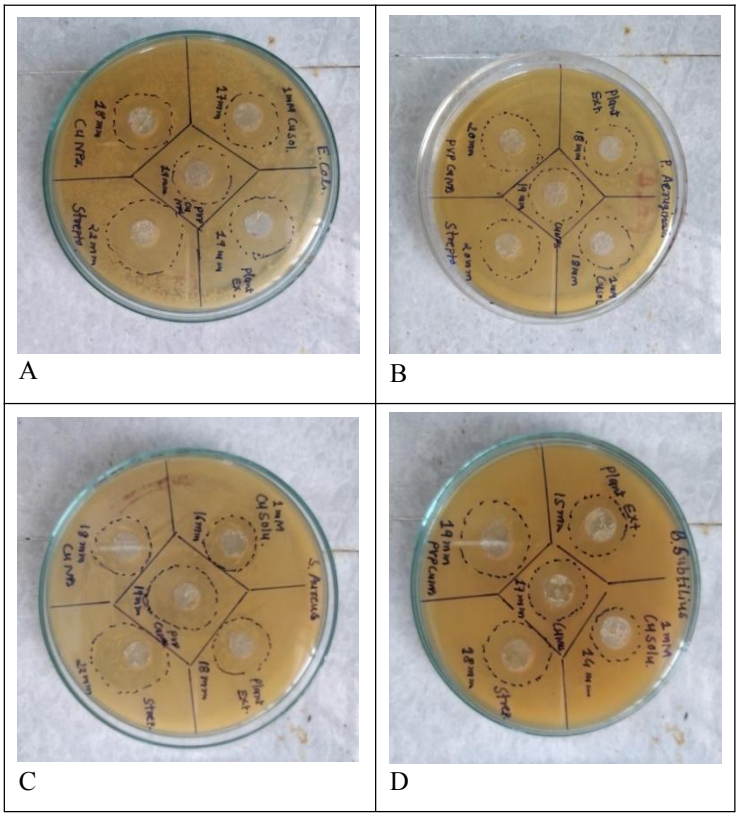

Figure 7. Antimicrobial study of biosynthesized CuNPs and PVP CuNPs against pathogenic bacteria [A] *Escherichia coli* [B] *Pseudomonas aeruginosa* [C] *Bacillus subtilis* [D] *Staphylococcus aureus.*

3.5. Antioxidant activity of CuNPs and polymer functionalized CuNPs

(1) 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method

The antioxidant potential of synthesized CuNPs and PVP-functionalized CuNPs was assessed using a DPPH free radical scavenging assay and compared against standard ascorbic acid across concentrations ranging from 10 to 100 µg/mL (Table 1). The results reveal that both nanoparticle formulations exhibit dose-dependent scavenging activity, with a notable enhancement upon PVP functionalization.

Table 1. Antibacterial activity of CuNPs and PVP CuNPs.

Sr.No.	Organism	Zone of Inhibition (In mm)				
		$CuSO_4$ Solution (1 mM)	Plant extract	CuNPs	PVP CuNPs	Streptomycin (1 mg/ml)
1	Escherichia coli	16	16	21	22	18
2	Staphylococcus aureus	14	15	15	18	19
3	Bacillus subtilis	14	15	18	19	16
4	Pseudomonas Aeruginosa	15	18	17	22	23

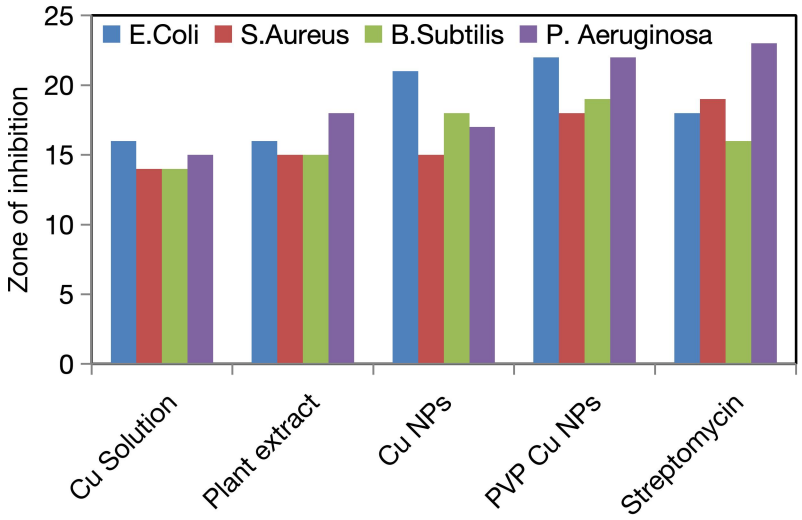

Figure 8. Antibacterial zone of inhibition of CuNPs and PVP CuNPs in comparison with standard streptomycin.

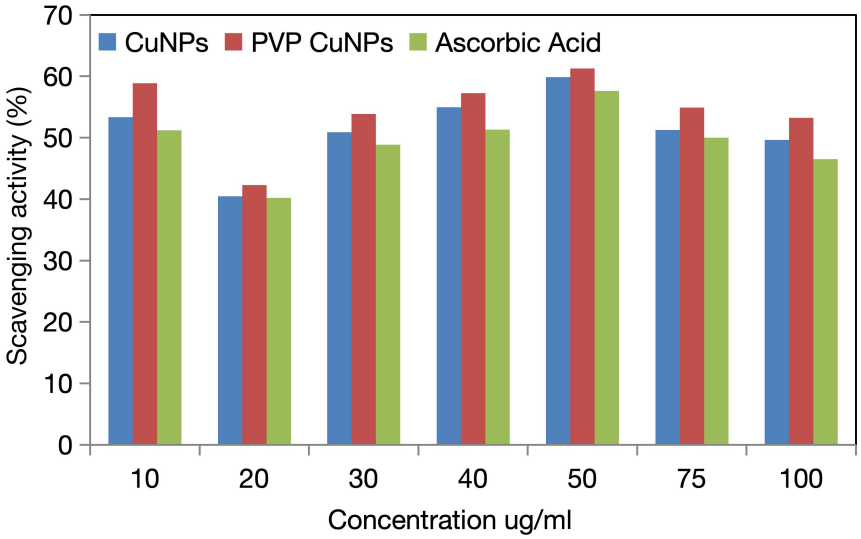

Figure 9. Antioxidant activity (%) of synthesized CuNPs and PVP CuNPs in comparison with standard ascorbic acid.

At lower concentrations (10–30 µg/mL), PVP-CuNPs exhibited higher scavenging activity than both CuNPs and ascorbic acid, indicating superior free radical neutralization efficiency at minimal doses. This suggests that PVP not only improves nanoparticle stability but may also contribute synergistically to antioxidant activity, possibly by facilitating better electron donation or enhancing radical interaction at the nanoparticle surface

At higher concentrations (40–50 µg/mL), both PVP-CuNPs and CuNPs achieved peak scavenging activity, with values reaching approximately 60%. Remarkably, this activity was comparable to or even slightly higher than that of ascorbic acid at the same concentrations, emphasizing the strong antioxidant capability of the nanoparticle systems.

Beyond 50 µg/mL, a slight decline in activity was observed for all samples, which may be attributed to saturation effects or potential agglomeration at higher concentrations, limiting effective interaction with DPPH radicals.

The overall trend demonstrates that PVP-CuNPs consistently outperform CuNPs and ascorbic acid, particularly at lower and moderate concentrations, highlighting the efficacy of PVP as a functionalizing agent in enhancing antioxidant behavior. These findings suggest that PVP-CuNPs hold significant promise as potent antioxidant agents, potentially useful in therapeutic applications targeting oxidative stress-related pathologies.[35]

4. Conclusion

In conclusion, this study successfully demonstrated the synthesis of copper nanoparticles (CuNPs) using an extract of Aradusi leaves and $CuSO_4 \cdot 5H_2O$ salt solution. Subsequently, the CuNPs were further functionalized with polyvinylpyrrolidone (PVP) to enhance biocompatibility, without the use of any harmful or toxic materials. The confirmation of CuNPs formation was validated by UV-visible spectroscopy, which exhibited a characteristic color change to dark brown and a peak at 247 nm after 24 hours. FTIR spectra analysis elucidated the various functional groups present in the Aradusi extract responsible for the biogenic synthesis of CuNPs and polymer-functionalized CuNPs. X-ray diffraction (XRD) examination confirmed the crystalline nature of the nanoparticles and revealed an average particle size of 70.20 nm for the polymer-capped CuNPs. High-resolution transmission electron microscopy (HR-TEM) imaging depicted spherical nanoparticles with sizes ranging from 10 to 100 nm. Moreover, both CuNPs and polymer-capped CuNPs exhibited significant antibacterial and antioxidant activities. Overall, this study highlights an environmentally friendly and cost-effective biological approach for synthesizing polymer-capped nanoparticles with potent antibacterial and antioxidant properties.

Authors Contribution

Dr. Faruk Arodiya: Conceptualization, Investigation, Visualization, Writing—Original Draft. Dr. Chirag Makvana: Supervision, Methodology, Validation, Formal Analysis, Reviewing & Editing. Miss Nahid Malik: Writing—Introduction, Editing. Dr. Kokila Parmar References, Editing & Formatting.. All authors have read and agreed with the published version of the manuscript.

Conflicts of Interest

There are no conflicts of interest reported by the writers.

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Data Availability statement

The data presented in this study are available on request from the corresponding author.

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