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RESEARCH ARTICLE

INNOVATIVE GREEN SYNTHESIS AND CHARACTERIZATION OF COW URINE-DERIVED CUPROUS OXIDE NANOPARTICLES FOR ANTI-MICROBIAL ACTIVITY

Imbabazizayo Prosper[1], Tuyizere Aime[1], Harshil Majethia[1], Said Salim Said[2]
[1]*Department of Pharmaceutical Science, Marwadi University, Rajkot, Gujarat, India.*
[2]*Zanzibar Food and Drug Agency (ZFDA), Zanzibar, Tanzania.*

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***Address for Correspondence:**
Said Salim Said, Zanzibar Food and Drug Agency, Zanzibar, Tanzania.
Tel: +255-777594373
E-mail: *saidalriyamy67@gmail.com*

Abstract

Background: The rise in bacteria resistant to antibiotics is a serious global health problem, pushing the search for new ways to fight them. Using green synthesis to synthesize nanoparticles is a sustainable alternative to traditional, more polluting methods. This research looks at using cow urine, a natural resource with components that can help in the synthesis, to create cuprous oxide (Cu_2O) nanoparticles.
Methods: Nanoparticles were synthesized through a simple green process that uses cow urine and ascorbic acid to reduce the starting materials. Then, we used several techniques UV-Vis spectroscopy, FTIR, and Zeta potential/DLS – to check the nanoparticles and find out their properties like size and stability. Finally, we tested how well the nanoparticles could kill/deactivate *Staphylococcus aureus* and *Escherichia coli* bacteria.
Results: The tests confirmed presence of Cu_2O nanoparticles, with a key absorbance at 419 nm. FTIR showed the presence of Cu-O bonds and other chemical groups. The nanoparticles were moderately stable, with a zeta potential of -4.732 mV, and their size was around 271.3 nm. They were effective against both bacteria, but *S. aureus* was more sensitive to them.
Conclusion: This study shows that it's possible to produce Cu_2O nanoparticles in a green and easy way using cow urine and ascorbic acid. These nanoparticles can effectively fight bacteria, suggesting they could be a valuable tool in the fight against antibiotic resistance and a more sustainable approach to making nanomaterials.
Keywords: Antimicrobial activity, ascorbic acid, cow urine, cuprous oxide nanoparticles, green synthesis.

INTRODUCTION

The domain of nanotechnology has experienced a notable transition towards environmentally sustainable synthesis methodologies. This shift is motivated by the imperative to mitigate environmental toxicity, decrease energy expenditure, and leverage renewable sources in the fabrication of nanoparticles[1,2]. Within the spectrum of metal oxides, cuprous oxide nanoparticles have attracted substantial scientific attention owing to their distinctive physicochemical characteristics and diverse applications spanning catalysis, biosensing, anti-microbial interventions, and Oncological thera-peutics[3,4]. Conventional synthesis techniques frequ-ently employ hazardous solvents and elevated temperatures, presenting ecological and biological hazards, thereby underscoring the necessity for the investigation of environmentally friendly synthesis strategies[5,6]. Contemporary research has increasingly concentrated on the utilization of biological substrates, encompassing plant extracts, microorganisms, and animal-derived materials, to facilitate the biogenic synthesis of nanoparticles[7]. An especially promising and relatively underexplored biogenic resource in this context is cow urine, which comprises a complex mixture of bioactive constituents, including urea, creatinine, phenolic compounds, enzymes, and a variety of inorganic minerals[8]. The aforementioned components of cow urine demonstrate inherent reducing, capping, and stabilizing capacities, positioning it as a sustainable and economically viable substitute for synthetic chemical precursors. In the present investigation, ascorbic acid was employed in conjunction with cow urine[9]. Ascorbic acid is a recognized environmentally friendly reducing agent, and its combined application with cow urine is hypothesized to augment the reduction kinetics and stabilization of Cu^{2+} ions, thereby facilitating enhanced nanoparticle synthesis[10]. The present study is designed to synthesize and characterize Cu_2O nanoparticles utilizing an optimized biogenic synthesis approach that incorporates cow urine and ascorbic acid, obviating the requirement for exogenous capping ligands such as cetyltrimethyl ammonium bromide or polyvinyl pyrrolidone[11]. Although prior investigations have explored the application of plant-derived materials in Cu_2O synthesis, the synergistic utilization of cow urine in conjunction with ascorbic acid in this process constitutes a largely uninvestigated area, thereby representing a novel contribution to the advancement of sustainable nanotechnology[12]. This research endeavor not only advocates for a cost-effective and ecologically sound methodology for nanoparticle formation but also valorizes a naturally occurring biological waste product, aligning with the fundamental tenets of green chemistry and the circular bioeconomy.

MATERIALS AND METHODS

Cuprous Oxide nanoparticles were synthesized based on the different studies with some modification. The Cu_2O nanoparticles were synthesized by starch-reduction approach. First, 0.1 M copper sulfate ($CuSO_4$) solution was mixed with 120 mL of 1.2% starch solution under continuous stirring of 400 rpm for 30 minutes in order to get homogeneous mixture. Secondly, 50 mL of 0.2M ascorbic acid solution, which was prepared using cow urine, was gradually mixed with the reaction mixture with continuous stirring. Ascorbic acid served as a reducing agent in the process of nanoparticle preparation. Thirdly, 30 mL of 1M sodium hydroxide solution was dropped in the mixture while continuously stirring. The whole reaction was conducted under continuous stirring conditions for 2 hours at 80°C to facilitate the synthesis of cuprous oxide nanoparticles. Once the reaction was completed, the solution was taken off heat source and kept overnight under room temperature conditions. Then, the solution was filtered to get cuprous oxide nanoparticles that were formed during the reaction. These nanoparticles were washed three times with deionized water and ethanol in order to get rid of the unreacted substances and impurities. Finally, the dried nanoparticles were subjected to oven-drying at 40°C for one hour followed by grinding them for 30 minutes using mortar and pestle[13].

Characterization of cuprous oxide nanoparticles: As nanomaterials are beyond the perception of human eyes, it becomes impossible to visualize its morphology and nature. Consequently, it becomes indispensable to analyze the synthesized substances utilizing sophisticated characterization methodologies. To date, no chemical analytical pathways are routinely employed; therefore, reliance on physical methods of analysis is necessary. Researchers commonly utilize physical techniques for the examination of nanomaterials. While physical analysis methods can be costly and necessitate skilled personnel for operation, contemporary instrumentation can precisely determine the dimensions of materials, and electron micrographs can elucidate the morphology of nanomaterials. Many advanced research institutions now possess these facilities. The array of characterization techniques includes ultraviolet-visible spectroscopy, X-ray diffraction, electron microscopy, zeta potential measurement, and dynamic light scattering, among others[14].

Antimicrobial assays: Antibacterial activity of synthesized Cu_2O nanoparticles was tested by employing agar well diffusion technique. Sterilization process was performed under the following conditions: liquid Mueller Hinton agar medium and Petri dishes were subjected to autoclaving in an autoclave machine at 121°C, 15 psi, for a period of 30 minutes. Following this, 4 mm thick layers of sterile agar medium were poured into Petri dishes. Prior to inoculation of agar medium, 18 hours' cultures of *S. aureus* and *E. coli* were evenly spread onto the surface of the agar plates. Wells were formed on agar plates using a sterile cork borer and different concentrations of Cu_2O nanoparticles and amoxicillin (control) were added to these wells[15].

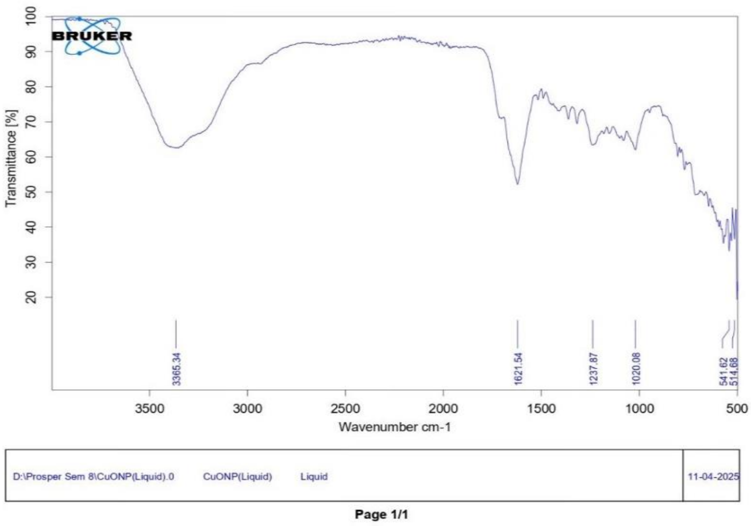

Figure 1: FTIR analysis of synthesized Cu_2O nanoparticles.

RESULTS AND DISCUSSION

The successful synthesis of copper oxide nanoparticles (nanoparticulated Cu_2O) was confirmed by the application of UV–visible and Fourier Transform Infrared Spectroscopy (FTIR). In particular, during the current research, the use of UV-Visible spectroscopy was performed, and a characteristic absorption peak was found at the wavelength of 419 nm. The latter corresponds quite well with the absorption range of Cu_2O nanoparticles, mentioned in scientific literature and proves the synthesis of the necessary semi-conductors. As it can be seen, an absorption peak at 419 nm describes the optical properties and electronic transitions of nanocrystalline copper oxide. At the same time, the results of FTIR analysis prove the successful synthesis of Cu_2O nanoparticles through a particular characteristic of the Cu-O bond stretch at 1020.08 cm^{-1}. Moreover, the presence of a wide absorption band at 3365.34 cm^{-1} is attributed to the O-H bond stretch, and it means the adsorption of water molecules from the atmosphere on the nanoparticles' surface[16]. Furthermore, the peaks at 1621.54 cm^{-1} and 1237.87 cm^{-1} are explained by the carbon-containing functional groups that might have been introduced into the sample in the process of synthesis from starch. In this way, the results prove that the molecules of starch were involved in the formation of nanoparticles[17].

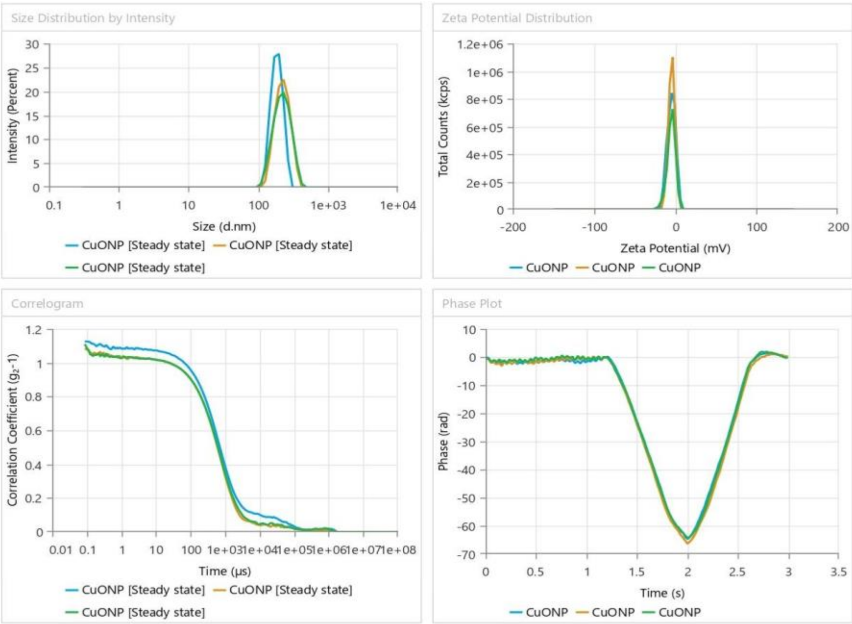

Figure 2: Particle size analysis of synthesized nanoparticles.

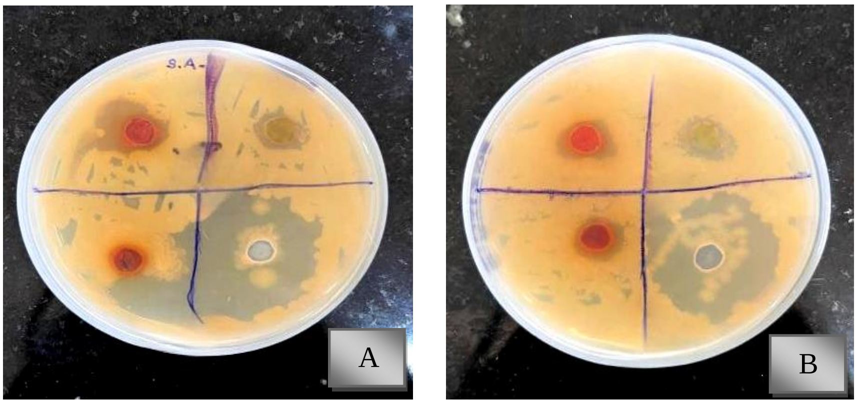

Figure 3: Inhibition of bacteria growth by Cu_2O NPs in (A): *S. aureus* and (B): *E. coli.*

Moreover, particle size and zeta potential analysis were carried out. Particle size analysis showed that the obtained nanoparticles had a hydrodynamic diameter of 271.3 nm with a relatively high degree of poly-dispersity. It might mean that the nanoparticles tend to aggregate, probably, because of poor electrostatic repulsion between particles[18]. At the same time, the relatively low zeta potential (-4.732 mV) indicates insufficient electrostatic repulsion between the particles. However, the obtained data still allows claiming that the nanoparticles demonstrate good colloid stability owing to the adsorption of starch molecules[19]. It is important to mention that a large hydrodynamic diameter seems unexpected. Based on the antibacterial properties of the prepared nano-particles, it was shown that the nanoparticles were capable of inhibiting the growth of both *S. aureus* and *E. coli*. Thus, the broad antibacterial properties of the produced nanoparticles were proven. Moreover, it was established that minimum inhibitory concentrations (MIC) against *S. aureus* were 3 mg/mL, while for *E. coli*, they were 10 mg/mL[20,21]. It means that Gram-positive bacteria were proven to be more sensitive than Gram-negative bacteria to the influence of Cu_2O nanoparticles, whereas Gram-negative strains were less sensitive. It may be attributed to the fact that the outer layer of the structure of Gram-negative bacteria decreases nanoparticle penetration and antibacterial activity[22]. Concerning the mechanism of the anti-bacterial properties of synthesized Cu_2O nanoparticles, it includes the release of copper ions (Cu^+ and Cu^{2+}), as well as the formation of ROS, resulting in oxidation. It causes the disruption of membranes, damages proteins and nucleic acids, and finally, leads to cell death[23]. In conclusion, the conducted research demonstrated the efficiency of the proposed starch-assisted method in obtaining nanoparticles with biological activity. Besides, it provided proof of the chemical and structural composition of nanoparticles and anti-bacterial activity. Using environmentally friendly stabilizers is an adequate solution to overcome disadvantages of traditional nanoparticle synthesis approaches. Despite the great number of previous studies on the impact of nanoparticles based on copper oxides, the present research provides valuable information regarding antibacterial properties of nanoparticles. Therefore, it can be concluded that it opens interesting perspectives for the usage of nanoparticles with starch in different fields. None-theless, further research is needed for evaluation of safety, efficacy, and possible practical applications of Cu_2O nanoparticles with starch.

CONCLUSIONS

This study successfully developed a green and scalable method for making cuprous oxide (Cu_2O) nanoparticles. The process used cow urine and ascorbic acid, offering a more sustainable alternative to traditional chemical synthesis by cutting down on the use of harmful chemicals and energy. The resulting Cu_2O nanoparticles showed good antibacterial activity against both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria, with *S. aureus* being more sensitive. These findings suggest that Cu_2O nanoparticles made through this eco-friendly approach could be useful as antibacterial agents to fight anti-biotic resistant bacteria. Moreover, this research points to the potential of using biological waste, like cow urine, to produce valuable nanomaterials, which fits well with the principles of green nanotechnology and a circular economy. Future research could explore the *in-vivo* applications of these nanoparticles and optimize the synthesis for even greater yield and stability.

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AUTHOR'S CONTRIBUTIONS

Prosper I: writing the original draft, methodology, investigation. **Aime T:** conceptualization, literature survey, data processing. **Majethia H:** editing, data curation. **Said SS:** methodology, investigation. Final manuscript was checked and approved by all authors.

DATA AVAILABILITY

The datasets generated or analyzed during this study are available from the corresponding author upon reasonable request.

CONFLICTS OF INTEREST

None to declare.

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