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Research Article

Green Synthesis of Copper Oxide Nanoparticles Using *Senna Siamea* Leaves Extract: Antibacterial Potential and Prospects for Sustainable Medical and Environmental Applications

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***Abstract*—**Nanoparticles have garnered significant attention due to their diverse applications in medicine, engineering, and environmental science. However, the chemical synthesis of nanoparticles often involves significant costs and environmental hazards due to the release of toxic byproducts. To address these challenges, we examined the environmentally sustainable and cost-effective biosynthesis of copper oxide nanoparticles (CuONPs) using Senna siamea leaf extract as a bioreducing agent. This research entailed the synthesis of copper oxide nanoparticles from copper sulfate ($CuSO_4$) in an aqueous solution via a green synthesis approach. The synthesized CuONPs were evaluated using UV-Visible spectroscopy and Fourier-transform infrared (FTIR) spectroscopy to evaluate their structural and morphological properties. The antibacterial effectiveness of CuONPs and Senna siamea leaf extract was assessed against Gram-positive bacteria (Staphylococcus aureus) and Gram-negative bacteria (Salmonella spp.). The results indicated significant antibacterial efficacy of the CuONPs, exhibiting greater effectiveness against Gram-negative bacteria compared to Gram-positive bacteria. This study highlights the potential of Senna siamea leaf extract as an environmentally sustainable alternative for nanoparticle production, as well as its possible antibacterial applications.

***Keywords*—** Copper oxide nanoparticles (CuONPs), Biosynthesis, Senna siamea leaf extract, Antibacterial activity, Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Salmonella* spp.), UV-Visible spectroscopy, FTIR spectroscopy.

1. Introduction

Nanotechnology has arisen as a swiftly progressing discipline with substantial applications across various areas, including medical, agriculture, environmental research, and engineering. Metal oxide nanoparticles have garnered significant attention among nanomaterials due to their distinctive physicochemical features and many uses. Copper oxide nanoparticles (CuONPs) have gained significance due to their antibacterial, antifungal, and catalytic properties [1]. Conventional nanoparticle synthesis methods frequently utilize toxic chemicals and high-energy procedures, resulting in environmental damage and elevated costs [2].

Recently, attention has turned to green synthesis methods that employ biological entities, including plant extracts, microbes, and enzymes, as reducing and stabilizing agents. These approaches are environmentally sustainable, economically viable, and offer a sustainable alternative to chemical synthesis [3]. Plant extracts, notably, exhibit significant potential in nanoparticle biosynthesis due to their abundance of bioactive chemicals such as alkaloids, flavonoids, phenols, and tannins, which promote nanoparticle formation [4]. CuONPs are notably appealing owing to their superior physicochemical characteristics, including elevated surface area, thermal stability, and economical synthesis, alongside their potential antibacterial efficacy [5]. The significant energy requirements of chemical synthesis highlight the necessity for more sustainable and ecologically friendly alternatives [6].

This research investigates the manufacture of CuONPs utilizing the leaf extract of Senna siamea, a medicinal plant recognized for its abundant phytochemical constituents and biological properties. The synthesis procedure employs copper sulfate ($CuSO_4$) as a precursor and leaf extract as a reducing agent in an aqueous media. The produced CuONPs were evaluated by UV-Visible spectroscopy, Fourier-transform infrared (FTIR) spectroscopy to ascertain their structural and morphological features. Additionally, the antibacterial efficacy of CuONPs and Senna siamea leaf extract was assessed against Gram-positive (Staphylococcus aureus) and Gram-negative (Salmonella spp.) bacteria.

This research emphasizes the dual benefits of employing plant extracts for nanoparticle synthesis: an eco-friendly technique and the generation of nanoparticles with notable biological activity. The results establish a basis for additional investigation into the synthesis of plant-based nanoparticles and their uses across multiple fields, including medicine, agriculture, and environmental cleanup.

2. Related Work

Prior investigations on the green synthesis of copper oxide nanoparticles (CuONPs) have illustrated the viability of environmentally sustainable methods for nanoparticle fabrication. [7] produced CuONPs with Punica granatum peel extract, mitigating the environmental issues associated with chemical production methods while demonstrating notable antibacterial and antioxidant effects. [8] investigated the biogenic production of copper nanoparticles utilizing Eclipta alba, emphasizing its effectiveness against multidrug-resistant bacteria. In a similar vein, [9] employed Azadirachta indica (neem) leaf extract to synthesize CuONPs exhibiting antifungal properties, with the objective of addressing the rising issue of fungus resistance. [10] utilized Ocimum sanctum extract to manufacture CuONPs, noting improved antibacterial and antioxidant activities, hence highlighting the biological potential of plant-mediated synthesis. [11] illustrated the efficacy of CuONPs derived from Moringa oleifera leaf extract in battling both Gram-positive and Gram-negative bacteria, hence aiding in the advancement of sustainable antibacterial agents. [12] emphasized the catalytic efficacy of CuONPs produced from Cymbopogon citratus for environmental remediation, demonstrating their capacity to breakdown organic contaminants. [13] produced CuONPs utilizing Allium cepa (onion) peel extract and demonstrated their dual antibacterial and anticancer properties, tackling significant healthcare issues. [14] examined the environmental applications of CuONPs produced from Citrullus lanatus peel extract, illustrating its efficacy in pollutant degradation. [15] developed CuONPs utilizing Mentha piperita extract to address antimicrobial resistance, demonstrating significant activity against drug-resistant bacterial strains. [16] utilized Eucalyptus globulus leaf extract for the synthesis of CuONPs, highlighting their significant antibacterial properties and ecological sustainability. These findings collectively underscore the increasing interest in plant-mediated nanoparticle production as an environmentally sustainable alternative, with applications spanning antibacterial drugs, environmental remediation, and medicinal innovations. Nevertheless, although these research offer significant insights, further investigation into novel plant species, such as Senna siamea, is necessary to optimize synthesis processes and improve biological activities. This study seeks to address this gap by utilizing Senna siamea leaf extract for the manufacture of CuONPs and assessing their antibacterial characteristics, thereby enhancing the overall comprehension of sustainable nanomaterials.

3. Experimental Method

3.1 Materials and Reagents

This study utilized analytical-grade copper sulfate ($CuSO_4$) as the precursor for synthesizing copper oxide nanoparticles (CuONPs) and fresh leaves of Senna siamea to make a leaf extract, which acted as a natural reducing and stabilizing agent. All solution preparations and washing procedures utilized distilled water. The characterisation of the produced CuONPs utilized a UV-visible spectrophotometer to analyze optical characteristics, Fourier Transform Infrared Spectroscopy (FTIR) to identify functional groups, and a Scanning Electron Microscope (SEM) to examine surface shape and size. Nutrient agar medium was formulated for bacterial culture, utilizing Staphylococcus aureus (Gram-positive) and Salmonella spp. (Gram-negative) as the test strains in the antibacterial activity assay. Petri dishes, sterile discs, and an incubator were employed for antibacterial testing, while pipettes, beakers, and additional glassware assisted in solution production. A centrifuge was utilized to extract CuONPs from the reaction mixture, and filter paper was applied for filtration. The ingredients and reagents facilitated the successful biosynthesis, characterisation, and assessment of the antibacterial efficacy of the CuONPs produced from Senna siamea leaf extract.

3.2 Extract Preparation

The freshly picked Senna siamea leaves were carefully washed in distilled water and tap water to get rid of dirt and other impurities, and then left to dry in the shade for a week. The dried leaves were ground into a powder and let to dry naturally at room temperature. Ten grams of powdered leaves and one hundred milliliters of distilled water were heated on a hot plate at one hundred degrees Celsius for thirty minutes to make the extract. Following filtration via Whatman filter paper, the extract was stored in a volumetric flask for future use.

3.3 $CuSO_4$ Solution Preparation

0.2 g of copper sulfate ($CuSO_4$) was precisely measured and dissolved in 100mL of distilled water in a beaker. The produced solution was thereafter transferred to a volumetric flask for future use.

3.4 Synthesis of CuO-NPs

To synthesize copper oxide nanoparticles (CuO-NPs), 10 mL of Senna siamea leaf extract was incrementally introduced to 90mL of 0.2M $CuSO_4{\cdot}5H_2O$ solution while maintaining continuous stirring on a magnetic hot plate. The incremental introduction of the extract resulted in a color transformation to brownish, signifying the synthesis of CuO-NPs. The solution underwent centrifugation for 10 minutes, after which the supernatant was discarded. The resultant viscous layer of CuO nanoparticles was collected, dried in an oven at 45°C for 24 hours, and the pellet was retrieved for subsequent examination.

3.4 Ultra Violet-VIsible spectroscopy
The produced CuO nanoparticles were evaluated with a UV-Visible spectrophotometer to assess their optical characteristics. The sample was positioned in a quartz cuvette, utilizing deionized water as the reference for baseline correction. The absorption spectrum was obtained in a wavelength range of 200–800nm at a uniform scanning velocity. The recorded spectrum indicated the surface plasmon resonance (SPR) peak, validating the effective synthesis of CuO-NPs and elucidating their optical properties.

3.5 FTIR Analysis
In order to determine which phytochemical functional groups were responsible for producing and stabilizing the dry, pure CuO nanoparticles, FTIR analysis was carried out. We placed a small amount of the sample on the FTIR sample holder and recorded its spectra between 400 and 4000cm^{-1}. The presence of phytochemicals in nanoparticle formation was confirmed by the FTIR spectrum, which revealed the chemical bonds and functional groups involved in the interaction between the leaf extract and CuO-NPs.

3.6 Preparation of Inocula
Bacillus spp., Staphylococcus aureus, and Salmonella spp. bacterial strains were isolated from culture plates and standardized to a turbidity level that corresponds to a 0.5 McFarland standard. For future antibacterial testing, the standardized bacterial suspensions were diluted in new peptone water and incubated at 36°C for 24 hours to obtain the final inoculum concentration.

3.7 Serial Dilution
For the preparation of serial dilutions, 2mL of the CuONPs solution was utilized as the initial stock. One milliliter of this stock was placed into a tube holding one milliliter of sterile distilled water. The procedure was conducted for four dilution steps, with each consecutive tube holding increasingly diluted CuONPs solutions. The resultant dilutions were employed for antibacterial testing.

3.8 Discs Preparation
Filter paper was perforated using a punch to produce discs of roughly 6 mm in diameter. The discs were subsequently autoclaved at 121°C for 15 minutes to guarantee sterility. Subsequent to sterilization, the discs were immersed in each concentration of the CuONPs solution and permitted to absorb the solution for one hour prior to antibacterial testing.

3.9 Sensitivity Test Using Disc Diffusion Method
Each bacterial inoculum was evenly placed onto the solidified Mueller Hinton Agar (MHA) plates using 0.2mL of the 39g sample. The plates were left to cool to room temperature until they stabilized. The contaminated agar surface was covered with sterile filter paper discs that had different concentrations of CuONPs (100mL, 75mL, 50mL, and 25mL), while the positive control consisted of discs that contained ciprofloxacin. The next step was to incubate the plates at 37°C for a full day. After the CuONPs were incubated, their antibacterial efficiency was assessed by measuring the diameter of the inhibitory zones surrounding each disc.

3.10 Preparation of McFarland Standard
The bacterium was cultured in sterile normal saline. After adjusting the concentration to achieve an optical density of 0.5, the benchmark for bacterial inoculum preparation in antimicrobial testing—the culture's optical density (O.D.) was measured at 600nm. To ensure that the sensitivity test used a consistent bacterial inoculum, the McFarland standard was used.

3.11 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC)
A dilution tube was used to determine the Minimum Bacterial Concentration (MBC) and Minimum Inhibitory Concentration (MIC). Testing tubes were filled with varying volumes of CuONPs extract (200mL, 100mL, 50mL, and 25mL) and then 0.1mL of the standardized bacterial inoculum was added to each. A 24-hour aerobic incubation period was conducted on the tubes at 37°C. In a separate experiment, we also established positive controls by cultivating the same types of bacteria but without the CuONPs extract. By subculturing the extract onto agar plates, we were able to identify the MBC as the lowest concentration at which no visible bacterial growth occurred, and the MIC as the lowest concentration at which no such growth occurred.

4. Results and Discussion

Uv-Visible Absorbance Studies
The reduction of copper ions (Cu^{2+}) to copper oxide nanoparticles (CuO-NPs) was clearly observed through a color change, which is attributed to the surface plasmon resonance (SPR) phenomenon. This phenomenon occurs due to the collective oscillation of free electrons in metal nanoparticles when they interact with light, producing an absorption band in the UV-Visible spectrum. The color change from sky blue to deep brown in the CuONPs samples is a direct indication of the reduction of Cu^{2+} to CuO-NPs, which is a key signature of nanoparticle formation.

The UV-Visible absorbance spectrum of the synthesized CuO-NPs showed several characteristic peaks. The first peak appeared at 205.01nm with an absorbance of 4.18A, followed by a peak at 224.57nm with an absorbance of 3.95A, and another at 235.83nm with an absorbance of 3.98A. A broader peak was observed at 381.67nm with a lower absorbance of 0.75A, which corresponds to the SPR band of the CuO-NPs. In contrast, the $CuSO_4$ precursor showed peaks at 215.13nm (absorbance 3.41A), 229.58nm (absorbance 3.38A), 221.10nm (absorbance 3.38A), and 208.54 nm (absorbance 3.38A), indicating the absence of nanoparticle formation. These differences in absorption peaks further confirm the successful synthesis of CuO-NPs, as the absorption at higher wavelengths (around 380nm) is typically associated with the SPR of copper oxide nanoparticles [17]. The presence of multiple peaks in the UV-Visible spectra of CuO-NPs further suggests the synthesis of nanoparticles with varying sizes and shapes, as different surface plasmon resonances can arise depending on these characteristics [18].

The observed results correlate well with findings from similar studies on the synthesis of metal oxide nanoparticles, where UV-Visible spectroscopy has been used as an effective tool to confirm nanoparticle formation, shape, and size distribution [19]. The peaks in the UV-Visible spectra serve as a reliable indication of nanoparticle synthesis and provide insights into their optical properties, which can further be used for applications in various fields such as antibacterial activities, catalysis, and sensors.

FTIRAnalysis

FT-IR spectroscopy was utilized to determine the functional groups linked to the active constituents in the leaf extract, $CuSO_4$ solution, and produced CuO nanoparticles (CuONPs). The FT-IR spectra of the three samples are displayed in the figure below. The spectra provide crucial insights into the chemical compositions and interactions pertinent to nanoparticle production.

Figure 2(a) illustrates the FT-IR spectra of the Senna siamea leaf extract, exhibiting distinctive peaks at 3280 cm^{-1}, 2922cm^{-1}, 1602cm^{-1}, and 1028cm^{-1}. The broad band at 3280cm^{-1} is ascribed to the stretching vibration of the O-H group, signifying the presence of hydroxyl groups, presumably from phenolic chemicals. The signal at 2922 cm^{-1} corresponds to the C-H stretching vibrations characteristic of the alkyl groups in phenolic compounds. The absorption peak at 1602 cm^{-1} results from the stretching vibrations of C=C, C-C, and C-O bonds in aromatic rings, indicating the presence of aromatic chemicals in the leaf extract. The signal at 1028 cm^{-1} corresponds to C-O stretching, presumably from amino acids and other organic compounds in the extract. Figure 2(b) displays the FT-IR spectra of the $CuSO_4$ solution, featuring peaks at 3332cm^{-1}, 2117cm^{-1}, and 1640 cm^{-1}. The broad band at 3332cm^{-1} is ascribed to O-H stretching, signifying the presence of alcohol groups or water molecules in the solution. The peak at 2117cm^{-1} is attributed to C-N stretching vibrations, potentially resulting from residual nitrogen-containing groups in the solution. The band at 1640cm^{-1} corresponds to the bending vibration of C=C bonds in aromatic rings, so proving the existence of aromatic chemicals in the solution.

Figure 2(c) illustrates the FT-IR spectrum of the synthesized CuONPs, exhibiting peaks at 3265cm^{-1}, 1595cm^{-1}, and 1058cm^{-1}. The broad band at 3265cm^{-1} corresponds to the stretching vibration of the O-H group, so proving the existence of hydroxyl groups on the nanoparticle surface. The peak at 1595cm^{-1} corresponds to the bending vibrations of C=C, C=O, and C=N bonds, suggesting the participation of phenolic compounds and other functional groups in the stability of CuONPs. The signal at 1058cm^{-1} is ascribed to C-N stretching, indicating the presence of amine groups, presumably from the amino acids or proteins in the plant extract that facilitated the reduction and capping of the nanoparticles.

The FT-IR study distinctly demonstrates the participation of many functional groups from the Senna siamea leaf extract in the production of CuONPs. The existence of hydroxyl, amine, and phenolic groups, coupled with the alterations in spectrum peaks pre- and post-nanoparticle synthesis, substantiates the reduction of Cu^{2+} ions to CuO-NPs and their stability via interactions with the active constituents of the plant extract. These results align with prior research on the production of metal oxide nanoparticles, indicating that plant extracts possess diverse functional groups that can reduce metal ions and stabilize the resultant nanoparticles [20].

Antibacterial Activity

The antibacterial potential of copper oxide nanoparticles (CuONPs), synthesized using Senna siamea leaf extract and $CuSO_4$ solution, was evaluated against two bacterial strains: the gram-positive *Staphylococcus aureus* and the gram-negative *Salmonella spp*. Ciprofloxacin was used as a positive control to compare the antibacterial activity of the synthesized nanoparticles. The results of the antibacterial testing are presented in Figures below and the corresponding values are shown in Tables 1 and 2.

The antibacterial activity of CuONPs was found to be dose-dependent, with a noticeable increase in activity as the dosage of nanoparticles was increased. This is consistent with previous studies, which suggest that the antibacterial efficacy of nanoparticles is influenced by both the concentration and particle size [21]. Specifically, CuONPs exhibited significantly higher antibacterial activity against *Salmonella spp.*, a gram-negative bacterium, compared to *Staphylococcus aureus*, a gram-positive bacterium. This higher susceptibility of gram-negative bacteria to CuONPs can be attributed to the structural differences between gram-positive and gram-negative bacterial cell walls. Gram-negative bacteria have a thinner peptidoglycan layer and an additional outer membrane, which makes them more prone to damage from nanoparticles [22].

In addition to the CuONPs, the leaf extract of Senna siamea also exhibited antibacterial properties, with stronger activity observed against *Salmonella spp.* than against *Staphylococcus aureus*. This suggests that the bioactive compounds present in the leaf extract may contribute to the antibacterial effects, with phenolic compounds, flavonoids, and other secondary metabolites potentially playing a key role in inhibiting bacterial growth [23].

The comparative antibacterial activity of CuONPs and the leaf extract underscores the potential of utilizing both plant-based synthesis methods and plant extracts themselves for antibacterial applications. Furthermore, the enhanced activity of CuONPs against gram-negative bacteria highlights their potential as effective antimicrobial agents in various biomedical and environmental applications.

Figure 1, Senna Siamea leaf

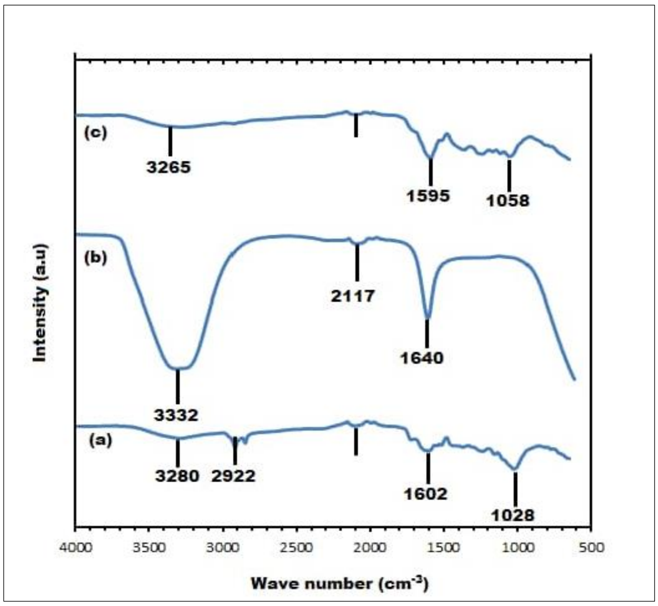

Figure 2, FTIR spectra of (a) senna siamea leaves extract, (b) $CuSO_4$, and (c) CuONPs

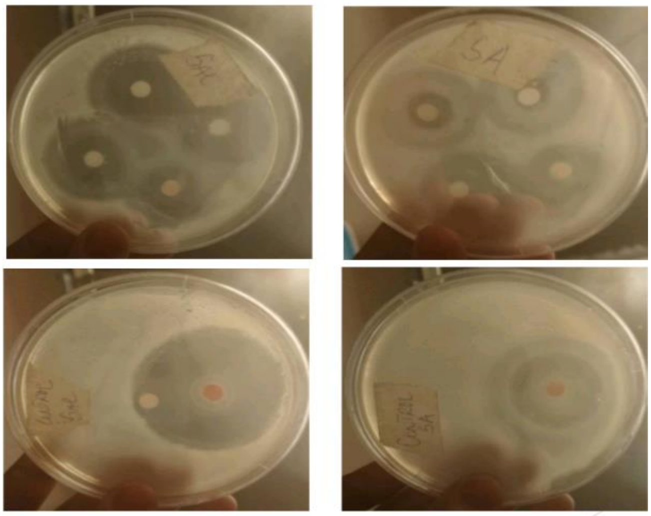

Figure 3, Antibacterial activity of CuONPs

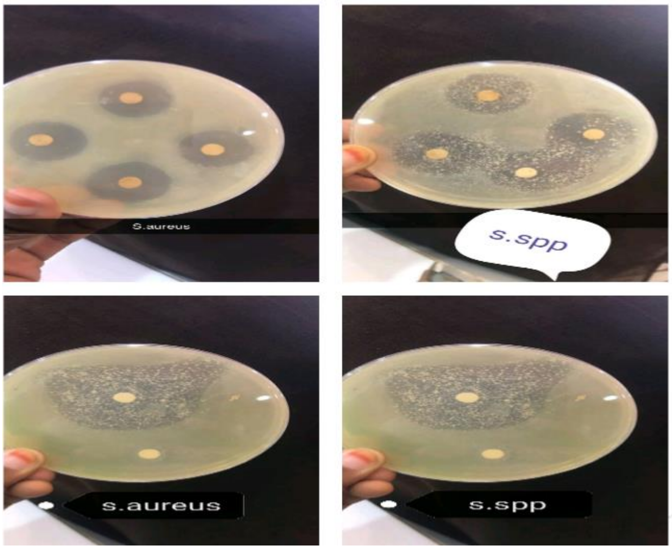

Figure 4, Antibacterial activity of leaf extract

TABLE 1, Bacterial activity values of leaves extract (*senna siamea*)

Bacteria	**100%**	**75%**	**50%**	**25%**	**Control**
S.aureus	23	20	19	17	35
S.spp	29	25	23	20	48

TABLE 2, Bacterial activity Values of CuONPs

Bacteria	**100%**	**75%**	**50%**	**25%**	**Control**
S.aureus	30	30	29	22	39
S.spp	32	33	28	20	49

5. Conclusion and Future Scope

The biosynthesis of copper oxide nanoparticles (CuONPs) using *Senna siamea* leaves extract and copper sulfate ($CuSO_4$) solution provides an environmentally friendly, simple, and cost-effective method for nanoparticle synthesis. The biosynthesized CuONPs were characterized by UV-Vis spectroscopy, which confirmed the formation of nanoparticles with a characteristic absorbance within the 200–800 nm range. The observed absorption peak corroborates the presence of CuONPs, further supported by Fourier Transform Infrared (FTIR) analysis, which identified functional groups in the *Senna siamea* leaf extract that play a crucial role in nanoparticle stabilization and capping.

The synthesized CuONPs exhibited significant antibacterial activity against both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Salmonella* spp.) bacteria, with enhanced efficacy observed against Gram-negative strains. This highlights the potential of *Senna siamea* leaf extract as a green reducing and stabilizing agent for nanoparticle synthesis and the promising antimicrobial properties of the resulting CuONPs.

Future studies should explore the optimization of synthesis parameters to achieve better control over nanoparticle size and shape, thereby enhancing their antimicrobial efficacy. Furthermore, expanding the spectrum of bacterial strains and investigating other biomedical applications, such as antifungal or antiviral properties, could provide deeper insights into the practical applications of biosynthesized CuONPs. The integration of these nanoparticles into medical and environmental technologies offers a sustainable approach to addressing global challenges in healthcare and environmental protection.

Data Availability
Because of the technical and time limitations, the raw data required as part of an ongoing study cannot be shared.

Conflict of Interest
The authors have no any potential conflicts of interest to declare.

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The authors do not receive any financial assist from any agency or institution for the research.

Authors' Contributions
The author Ibrahim Usman Gafai[1]* and Asiya Junaid[3] carried out the research and drafted the manuscript. Dr. Aminu Musa[3] supervised the research and edited the manuscript. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

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Ms. Asiya Junaid earned her Bachelor of science degree with second class upper in chemistry from Umaru Musa yar'adua university Katsina, Nigeria in the year 2023.During her undergraduate studies,she worked and gained experience with production unit Katsina youths craft in the year 2019 and 2020,as a part of students industrial work experience (SIWES), Additionally,she served as an office Assistant atDigibit control Nigerian Limited, during her National youth service corps (NYSC) program from 2023-2024,and a member of chemical society of Nigeria (CSN).she has 1year research experience,and has research interest in innovative chemical science, food chemistry, material science and polymer chemistry.

