Educational Administration: Theory and Practice
2024, 30(1), 3142-3148
ISSN:2148-2403
https://kuey.net/

Research Article

Green Synthesis Of Manganese Dioxide And Copper Oxide Nanoparticles Using Phaseolus Lunatus Flower Extract And Evaluation Of Their Antimicrobial Activity

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Citation: Sunil Ramrao Mirgane et.al (2024), Green Synthesis Of Manganese Dioxide And Copper Oxide Nanoparticles Using Phaseolus Lunatus Flower Extract And Evaluation Of Their Antimicrobial Activity, *Educational Administration: Theory and Practice*, *30*(1), 3142-3148
Doi: 10.53555/kuey.v30i1.7056

ARTICLE INFO

ABSTRACT

Manganese dioxide nanoparticles (MnO_2-NPs) and copper oxide nanoparticles (CuO-NPs) with suitable surface chemistry possess various beneficial properties that can be used in numerous biomedical applications, including magnetic resonance imaging contrast enhancement, tissue regeneration, hyperthermia, drug delivery, and cell separation. The study discovered that undiscovered Phaseolus Lunatus flower extract can effectively synthesize manganese dioxide nanoparticles (MnO_2-NPs) and copper oxide nanoparticles (CuO-NPs). The nanoparticles were analyzed by UV-visible spectrophotometer, SEM, EDS, XRD, and FT-IR to determine their properties. The synthesized MnO_2-NPs and CuO-NPs were naturally stabilized, cubic in shape, and sized between 26.98 nm and 65.20 nm. The phytochemicals in the leaf act as reducing agents, aiding in the environmentally friendly synthesis of MnO_2-NPs and CuO-NPs with improved antibacterial properties. The functional groups found on the nanoparticles are primarily -OH and -COOH, as indicated by FT-IR analysis, which makes them hydrophilic. Therefore, no additional functional modifications are required for their uses. The synthesized MnO_2 and CuO were tested for their antibacterial activity against Gram-negative (E. coli and P. aeruginosa) and Gram-positive (S. aureus and B. subtilis). The zone of inhibition measured 17 mm for E. coli and 15 mm for S. aureus. Naturally stabilized Gram-negative nanoparticles with herbal properties can be used in a variety of biological applications.

Keywords: MnO_2-NPs, CuO-NPs, Green synthesis, Phaseolus Lunatus, Antimicrobial activity

1. INTRODUCTION

Using plant flower extracts for synthesizing manganese and copper oxide nanoparticles provides eco-friendly and compatible options for pharmaceutical and biomedical applications since they avoid the use of harmful chemicals in the synthesis process [1-2]. Chemical synthesis processes can introduce hazardous compounds that may have negative effects on medical applications. Currently, the biological production of nanoparticles using plant extracts is being utilized by researchers [3,4]. There are numerous physical, chemical, biological, and hybrid ways of synthesizing various types of nanoparticles [5]. Each approach produces nanoparticles with distinct features. Plant-mediated production of metal nanoparticles is currently in progress [6-7]. Green nanotechnology is a field that has garnered significant interest and encompasses many methods aimed at minimizing or eradicating harmful compounds to help improve the environment. The manufacture of metal nanoparticles using inactive plant tissue, plant extracts, exudates, and other living plant components is a contemporary method. Green synthesis of nanoparticles uses environmentally friendly, non-toxic, and safe chemicals [8-12].

Plants and microbes produce inorganic materials that are mostly found in nanoscale sizes. Cellular extracts from various biological entities can produce nanoparticles with varying sizes and chemical contents [13-15]. Using various plant parts, predominantly leaves, for the extraction of metal nanoparticles is an efficient and cost-effective method known as green synthesis. Metal ions undergo bio-reduction throughout the synthesis process. The plant extract components reduce iron ions, while water-soluble heterocyclic components stabilize the nanoparticles. Metal salts can be used as a suitable precursor for reducing plant extracts. Green nanotechnology is a field that has garnered significant interest and involves many methods aimed at minimizing or eradicating harmful compounds to improve the environment.
Phaseolus lunatus is a big, softly hairy, round, climbing, or trailing plant found in India and elsewhere [16]. The entire plant is acknowledged to have medicinal properties in traditional medical systems. The fruit is tasty and possesses diuretic, antipyretic, antibilious, tonic, vulnerary, and antiperiodic properties. It can treat blood disorders, muscle soreness, and dry cough. Their phytochemicals contain hydroxyl, carboxyl, and amino functional groups. These groups can act as efficient metal-reducing agents and capping agents, creating a strong coating on metal nanoparticles in a single process. The manufacture of metal nanoparticles using deactivated plant tissue, plant extracts, exudates, and other components of living plants is a contemporary method [17]. Green synthesis of nanoparticles ensures the use of environmentally friendly, non-toxic, and safe chemicals. MnO_2-NPs and CuO-NPs nanoparticles have diverse biomedical applications, including serving as magnetic beads for trapping bacteria, creating sensors to detect different biological threat agents, and playing a crucial role in the medical field. We have examined the synthesis method of Mn and Cu nanoparticles using green chemistry. This study involved synthesizing iron nanoparticles by using dried powder from Phaseolus lunatus flower. MnO_2 and CuO nanoparticle production is summarized due to its significance in industry and the environment. Scientific research has confirmed the pharmacological effects of Phaseolus lunatus mentioned in Ayurvedic texts [18-19]. Studies have shown that the plant exhibits strong antioxidant, diuretic, antihyperglycemic, anticancer, analgesic, and antidepressant properties. This work examines the characterization and production methods of iron nanoparticles and evaluates their antibacterial efficacy. The MnO_2-NPs and CuO-NPs nanoparticles were synthesized by using potassium permanganate and copper chloride as the manganese and copper sources and Phaseolus lunatus flower extract as the reducing agent and stabilizer.

2. EXPERIMENTAL

2.1. Collection of Plant Material and Preparation of Extract
Flowers of Phaseolus lunatus were gathered from Local farmers in, the district of Maharashtra, India. The fresh flowers were used for all testing methods.

2.2. Chemicals
The investigation used potassium permanganate ($KMnO_4$) and copper chloride hexahydrate ($CuCl_2 \cdot 6H_2O$, 98%) and a solvent of the highest purity and analytical grade obtained from S. D. fine chem.

2.3. Extraction Preparation
The Phaseolus lunatus flower components were cleaned and kept at a temperature of -4 °C. To produce the extract, about 5 g of ground, air-dried Phaseolus lunatus samples (flower) were boiled with 100 ml of double-distilled water in an Erlenmeyer flask while being agitated constantly for 15 minutes. The extract was cooled to ambient temperature, filtered, and then stored at -4°C for future use.

2.4. Preparation of MnO_2-NPs
Manganese dioxide nanoparticles were prepared using an adjusted procedure based on earlier research studies [20-25]. Simply, mix a 0.01 M solution of $KMnO_4$ with the Phaseolus lunatus flower extract in a 1:1 volume ratio. MnO_2-NP nanoparticles were promptly prepared using the reduction procedure. The mixture was agitated for 60 minutes and then left at room temperature for an additional 30 minutes to achieve a colloidal suspension. The mixture was centrifuged, washed with ethanol multiple times, and then dried at 40 °C under a vacuum to produce the MnO_2-NPs. Flowers of Phaseolus lunatus have superior reduction capability against $KMnO_4$, as indicated by the exterior color change. Flowers meeting the criteria were chosen for additional processes. Following the confirmation test, the MnO_2-NPs were synthesized using the same process for additional characterization.
Preparation of CuO-NPs
A modified protocol based on previous research work was used to manufacture copper oxide nanoparticles [26–28]. To begin, just combine the Phaseolus lunatus extract in a 1:1 volume ratio with a 0.01 M solution of $CuCl_2 \cdot 6H_2O$. Rapid production of CuO nanoparticles was achieved by the reduction process. To obtain a colloidal suspension, the liquid was stirred for 60 minutes and then allowed to stand at room temperature for a further 30 minutes. To prepare the CuO nanoparticles, the mixture was centrifuged, repeatedly cleaned with ethanol, and vacuum-dried at 40 °C. The external color shift indicates that the Phaseolus lunatus flower have a higher reduction capability against copper chloride than other plant components (seeds and fruit). Flowers

that satisfied the requirements were selected for the next steps. The CuO-NPs were generated using the same procedure for further characterization after the confirmation test.

2.5. Characterization techniques

Characterization techniques aid in accurately and rapidly understanding the unique properties of substances or nanocrystals being researched, providing reliable insights into the measured values. The synthesized MnO_2 and CuO nanoparticles underwent various characterization studies to analyze specific properties including structural, morphological, elemental composition, particle size, and functional groups. These properties were precisely examined using advanced techniques such as UV-VIS spectroscopy (JASCO V650 UV-Vis model), XRD (PANalytical X'Pert Pro instrument with Cu Kα1 radiation of wavelength (λ) of 1.5406 (°A)), SEM, (FEI Nova Nano SEM 450) and EDS (Bruker XFlash 6I30) and FT-IR (BRUCKER). The strategies were useful in confirming that our method is well-optimized and meets the requirements.

2.6. Antimicrobial Activity Test

The antimicrobial ability of synthesized MnO_2 and CuO nanoparticles was examined to investigate the herbal functionality of nanoparticles.
Antimicrobial activity was assessed using the disc diffusion method, as described in a previous study [29-30]. The organisms employed for the antibacterial test were: Gram-negative: *E. coli*; and Gram-positive. *Staphylococcus aureus* Standard drug reference - Streptomycin at a concentration of 20 μg/ml.

3. RESULTS AND DISCUSSION

A single-step process coats metal nanoparticles and causes a color change from yellowish brown to brownish black. The color shift confirmed the formation of MnO_2-NPs and CuO-NPs. The flowers of the Phaseolus lunatus plant exhibit a superior ability to generate MnO_2 and CuO nanoparticles compared to other components of the plant including seeds and fruits.

3.1. UV-visible spectra

The preface test compares the UV-visible absorption peak at 516 and 713 nm, showing plant detritus and protein-bound MnO_2-NPs and CuO-NPs respectively [31] (**Figure 1)**. The discrepancy observed may be due to chloride interference in the plant extract [32].

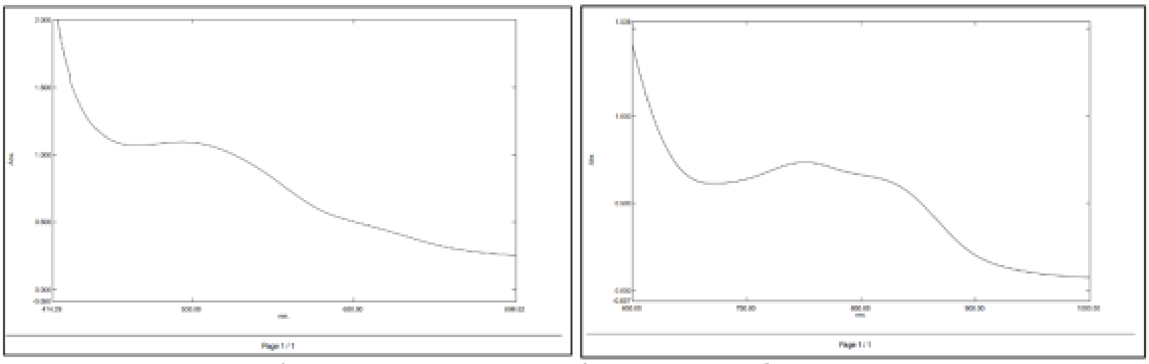

Figure 1. UV-vis spectra of MnO_2-NPs and CuO-NPs

3.2. EDX spectra

The EDX spectrum in **Figure 2** indicates the presence of chemicals such as Chloride, Calcium, and Potassium in the reaction mixture together with Mn and Cu nanoparticles, suggesting the formation of a complex with the plant extract.

The analysis showed that the flower extract from Phaseolus Lunatus contains 30.18% manganese and 34.19% oxygen in the total weight of MnO_2-NPs and 19.77% copper and 34.46% oxygen in the total weight of CuO-NPs. This is derived from the bremsstrahlung X-ray intensity concerning energy. Carbon alters the enzyme molecule's physical structure, revealing the suitable chemically active spots for reaction. Carbon neutralizes organic anions and other chemicals in the plant, stabilizing pH between 7 and 8, which is ideal for enzyme activity.
The concentration of carbon (C) in the cell dictates the number of enzymes that can be activated and the speed at which chemical processes can occur.

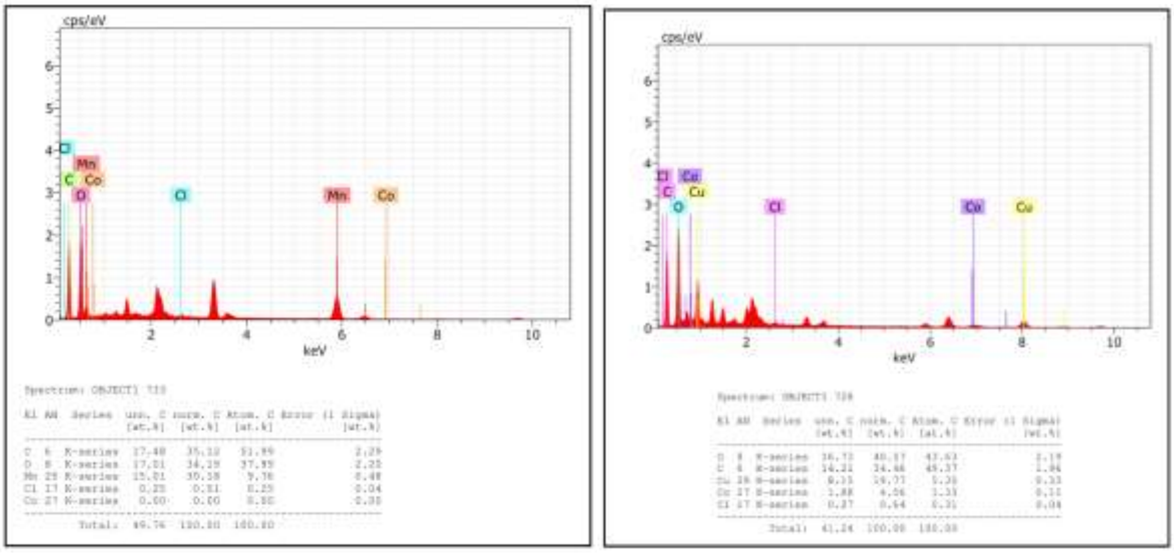

Figure 2: EDAX spectrum for MnO_2-NPs and CuO-NPs

3.3. SEM analysis:

The sample underwent SEM analysis for morphological examination and size measurement. The SEM was used to study the formation of MnO_2-NPs and CuO-NPs and their morphological dimensions. The study showed that the average size of the nanoparticles ranged from 26.98 nm to 65.20 nm, which aligns with findings from earlier studies. The production of cube-shaped manganese and copper nanoparticles is also demonstrated in **Figures 3** and **4**. The preparation of cube-shaped nanoparticles is caused by plant enzymes and chloride. The presence of carbon compounds in the sample influences the morphology of the nanoparticles. SEM micrographs provide a distinctive three-dimensional look that is beneficial for analyzing the surface structure of a material due to the narrow electron beam and great depth of field.

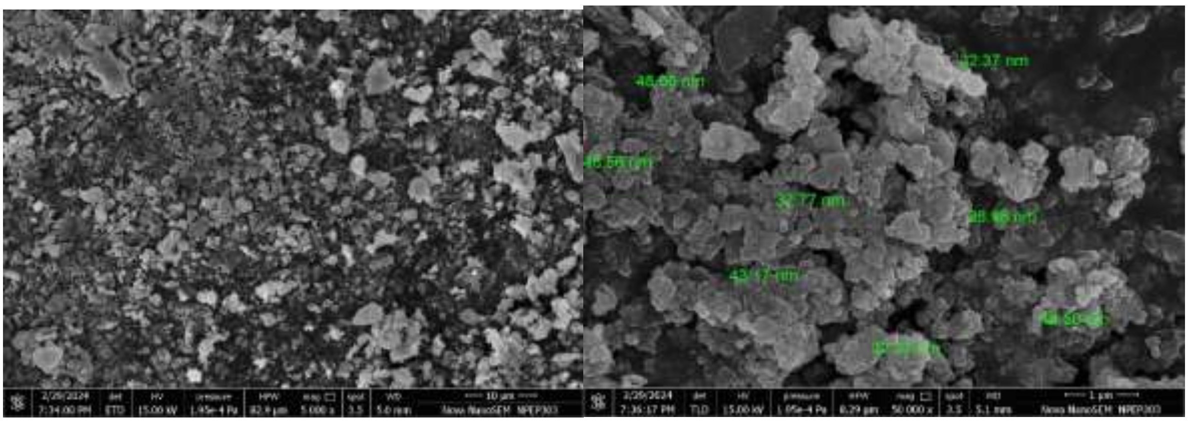

Figure 3: FE-SEM images of MnO_2-NPs with a magnification (10μm and 1μm)

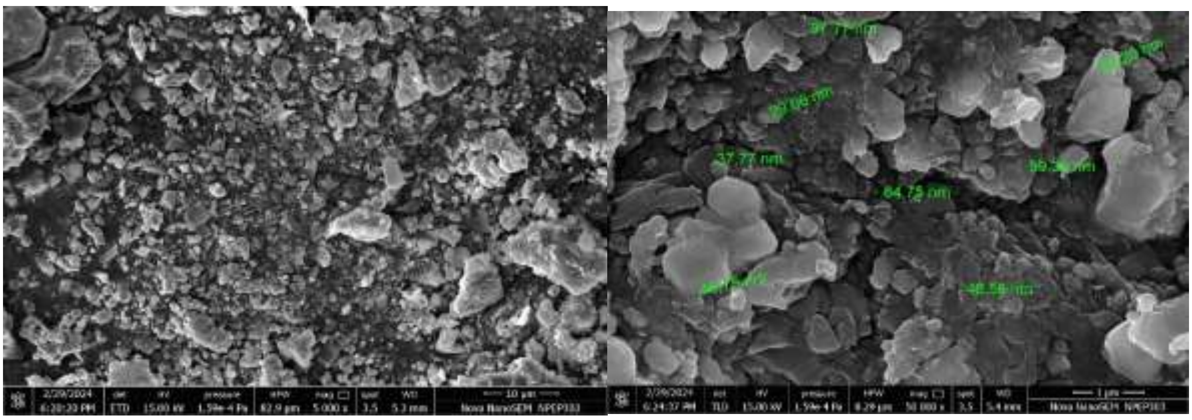

Figure 4: FE-SEM images of CuO-NPs with a magnification (10μm and 1μm)

3.4. X-ray diffraction spectra

Figure 5 displays the X-ray diffraction patterns of the MnO_2-NPs and CuO-NPs synthesized with Phaseolus lunatus extract. The X-ray diffraction analysis revealed strong diffraction peaks at 2θ values of 28.40° and 35.62°, corresponding to the hkl values of 220 and 222, indicating the crystalline phase of MnO_2-NPs and CuO-NPs nanoparticles matching JCPDS card No. 39-1346 and JCPDS card No. 89-4319. The grain size was determined using the Debye-Scherrer equation, establishing a relationship between peak broadening in XRD and particle size. The Scherrer equation was used to determine that the average crystallite sizes of the MnO_2-NPs and CuO-NPs fall within the range of 16 nm to 19 nm. The results showed that all the nanoparticles had a spinel structure with a face-centered cubic phase.

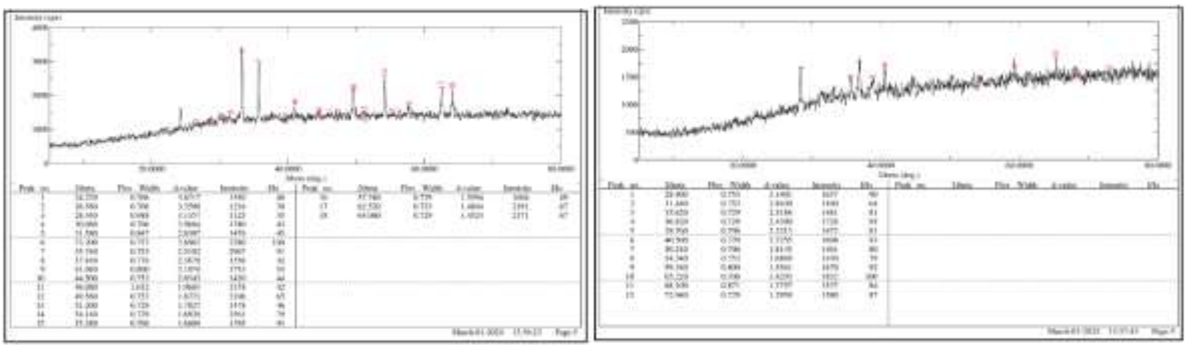

Figure 5: XRD spectra of MnO_2 and CuO NPs

3.5. FT-IR analysis:

FT-IR analysis identified stretching vibrations at 3329-3639 cm^{-1}, 1584-1587 cm^{-1}, and 613-617 cm^{-1} within the 500-4000 cm^{-1} area (**Figure 6**). The peaks indicate that the bonding in the sample validates the participation of the reducing agent in the creation of MnO_2-NPs and CuO-NPs. The peak at 3329-3639 cm^{-1} represents the -OH bond stretching in the aqueous phase and the reduction of metal salts. The peak at 1584-1587 cm^{-1} corresponds to the C=O bond stretching, indicating the presence of phytochemicals and amino acids that stabilize and act as capping agents in the plant extract. The remaining unexplained peaks indicate a minor number of organic acids that contribute to the low pH of the sample, facilitating the formation of the MnO_2-NPs and CuO-NPs. The prominent peak at 613-617 cm^{-1} corresponds to the inorganic stretching of MnO_2-NPs and CuO-NPs. The zeta potential of MnO_2-NPs and CuO-NPs was measured at -50 meV, indicating exceptional stability attributed to the significant negative surface charge.

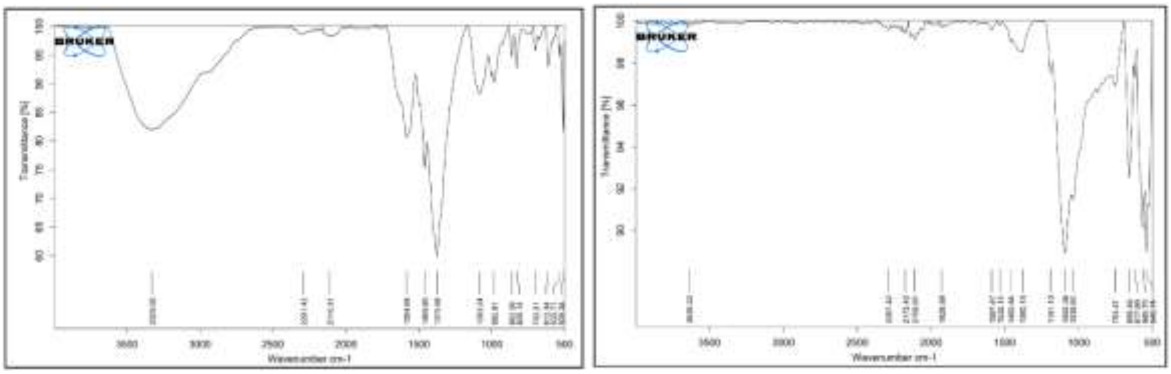

Figure 6: FT-IR spectra of MnO_2 and CuO NPs

The synthesized MnO_2-NPs and CuO-NPs exhibit moderate antimicrobial activity compared to the reference drug. They show higher antibacterial activity against E. coli and moderate activity against S. aureus. The inhibitory activities of the MnO_2-NPs and CuO-NPs in culture media are comparable to the standard antimicrobial Ampicillin.

Synthesized nanoparticles exhibit equivalent antibacterial efficacy compared to the original plant extract. The plant's herbal property-based functional groups covering the surface of NPs activate antibacterial capabilities. Reduced NP size enhances NP penetration into the cell wall, resulting in cell death. After conducting moderate activity, it was determined that E. coli was the more vulnerable bacteria and S. aureus was the more resistant. The MnO_2-NPs and CuO-NPs are naturally stabilized and possess a larger surface area suitable for numerous applications. The components involved in the production of MnO_2-NPs and CuO-NPs need to be isolated and purified to prevent the presence of plant contaminants that can affect the properties of the NPs.

3.6. Screening of antibacterial activity of manganese dioxide and copper oxide nanoparticles synthesized by Phaseolus lunatus flower extract

The antibacterial properties of MnO_2-NPs and CuO-NPs nanoparticles synthesized through green methods were evaluated against Gram-positive and Gram-negative bacterial strains using the agar well diffusion method. The results revealed that MnO_2-NPs nanoparticles significantly inhibited the growth of B. subtilis (22 mm) and E. coli (19 mm) at a concentration of 22 µg. In contrast, copper oxide nanoparticles exhibited moderate inhibition of E. coli (14 mm) and S. aureus (15 mm) at the same concentration. The inhibition zones produced by the commercial antibiotic ciprofloxacin (19 µg/disc) ranged from 10 to 19 mm, surpassing those generated by the nanoparticles. The concentration of synthesized MnO_2-NPs and CuO-NPs nanoparticles played a significant role in determining their antibacterial efficacy. These nanoparticles exhibited notable antibacterial activity against human pathogens such as E. coli, K. pneumonia, S. aureus, and B. subtilis.

4. CONCLUSION

The phytochemicals in the sample functioned as capping and reducing agents, naturally stabilizing the NPs. Despite having a lower nanoparticle yield compared to other chemical and physical processes, this method is preferred due to its non-toxic green synthesis and natural functionalization with herbal characteristics. The plant's herbal characteristics are demonstrated by its moderate antibacterial effectiveness against the selected pathogen. Only a small number of NPs can use the plant extract as a reducing agent for synthesizing NPs effectively. Future research will focus on validating the superparamagnetic for its application in cancer therapy.

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