https://doi.org/10.33472/AFJBS.6.11.2024.568

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

ANTIBACTERIAL EFFICACY OF BIOGENIC METAL-OXIDE NANOPARTICLES SYNTHESIZED USING MORINGA OLEIFERA LEAF EXTRACT

Dehbi Atallah[1*], Benchikh Imene[2], Dif Mustapha Mahmoud[3]

[1*,2]Department of Natural and Life Sciences, Institute of Sciences, Nour Bachir University Center, El-Bayadh 32000, Algeria
[2]Faculty of Science, University of Amar Telidji, Laghouat 03000, Algeria

Corresponding Email: [1*]dehbi.atallah@yahoo.com

Article Info

Volume 6, Issue 11, July 2024

Received: 22 May 2024

Accepted: 19 June 2024

Published: 08 July 2024

doi: 10.33472/AFJBS.6.11.2024.557-568

ABSTRACT:

Moringa oleifera leaf extract has garnered significant attention for its potential as a green reducing and stabilizing agent in nanoparticle synthesis. This biogenic synthesis route is cost-effective, eco-friendly, and free from toxic by-products. In this study, metal oxide nanoparticles, including copper oxide (CuO), zinc oxide (ZnO), silver oxide (AgO), and manganese oxide (MnO), were synthesized and characterized by using X-ray diffraction, scanning electron microscopy, and Fourier-transform infrared spectroscopy to determine their size, morphology, and chemical composition. The antibacterial activity of these nanoparticles was evaluated against pathogenic bacteria, demonstrating their potential for biomedical applications. Characterization confirmed the successful synthesis and crystalline structures of the nanomaterials. The average particle sizes of CuO, ZnO, AgO, and MnO nanoparticles were 44.70 nm, 27.04 nm, 37.11 nm, and 36.04 nm, respectively. Antibacterial assays revealed strong inhibitory effects against Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa, with the highest activity observed for Ag and CuO nanoparticles. Inhibition zones were measured up to 16.11 mm for E. coli and 14.8 mm for S. aureus. These results suggest that green-synthesized nanoparticles from Moringa oleifera extracts are promising antibacterial agents, offering a sustainable alternative to conventional antibiotics.

Keywords: Metals Oxide nanomaterials, Synthesized and characterized, Moringa oleifera leaf extract, Green synthesis, Antimicrobial activity.

1. INTRODUCTION

Antibiotic-resistant microorganisms evolve due to the overuse of antibiotics, which pose a major threat to public health worldwide. Antibiotic-resistant infections are responsible for about 800,000 deaths per year, with projections for up to ten million deaths per year by 2050 in the absence of effective interventions, according to the World Health Organization (Kraker et al., 2016). Standard treatments and preventive measures are becoming ineffective against resistant pathogens, which require developing new tools for treating these pathogens. Of these, metallic nanoparticles are particularly effective compounds due to their distinctive behavior, which results from their small size compared to more conventional bulk chemical materials and their high specific surface area (Álvarez et al., 2023). They are synthesized using chemical reduction (Marchianò et al., 2021), electrochemical methods (He et al., 2023), microwave-assisted techniques (Garadkar et al., 2016), and eco-friendly green chemistry processes for their stabilization.

Nanoparticles, as important agents in medicine and biology, have been studied since the 1980s, initially for their potential in drug delivery. Since then, advances in nanotechnology have ushered in countless applications, from imaging to diagnostics to therapy. Due to the differences in particle size, a large surface-to-volume ratio, and tunable morphological properties with respect to their bulk counterparts, nanomaterials have garnered increased interest, particularly those made from metal and metal oxide nanoparticles (Fifere et al., 2023). These nanoparticles enhance bactericidal activity through the production of reactive oxygen species (ROS), which affect the bacterial cell membrane, proteins, and DNA. They are small enough to enter bacteria and manipulate the cells' vital mechanisms. The nanoscale interactions and reactivity of metallic nanoparticles interfere with essential cellular processes and produce oxidative stress, ultimately leading to cell death, thereby contributing to the antibacterial properties of metallic nanoparticles (Shahzadi et al., 2018), The mechanism of this eco-friendly antimicrobial activity is illustrated in Fig. (1).

Yet, our incomplete knowledge of how nanoparticles exert their antimicrobial effects and their possible toxicity to human cells has restrained the clinical use of nanoparticles (Manuja et al., 2021). These challenges have led to a potential solution, which is the synthesis of metallic nanoparticles through green methods using natural plant extracts, offering a convenient eco-friendly pathway that can prevent harsh chemical use and minimize environmental impact (Mahabadi et al., 2021). Green synthesis of nanoparticles is achieved from a variety of biological matrices with reducing agents that convert metal ions into nanoparticles and bioactive molecules that confine and stabilize metal nanoparticles during synthesis (Rajak et al., 2023; Gnanasangeetha & Suresh, 2020). Extracts such as *neem*, *turmeric,* and *green tea* have been used to synthesize nanoparticles, reducing the environmental hazards of chemical synthesis and adding bioactive compounds inherent to the plants, thereby enhancing the bactericidal potential of the nanoparticles.

In this regard, among a variety of influential plant extracts in the reduction of metal ions into nanoparticles, plant extracts might be a more effective role by taking advantage of the availability and biodiversity of the plant species and the richness in plant secondary metabolites with reducing and stabilizing properties (Singh *et al*, 2018). *Moringa oleifera* leaf extract in synthesizing metal oxide nanoparticles has attracted the attention of scientists, using it as a reducing and stabilizing agent (Fifere et al., 2023). The plant which is popularly called as a Drumstick tree is famous for its nutritional and medicinal benefits. Due to the usage of prominent vitamins, amino acids, enzymes and many other natural antioxidants., its leaves are loaded with bioactive compounds Polyphenols, flavonoids, tannic acid, terpenoids, carbohydrates, amines, enzymes. These compounds are important in the reduction of metal ions to nanoparticles and their stabilization, which ensures the size is homogeneous, and the particles do not cluster together. In addition, biometallic nanoparticles synergize with these bioactive compounds to increases the antibacterial effect of the nanoparticles (Gautam et al., 2023).

In recent years, numerous studies have reported the environmentally friendly production of metal and metal-oxide nanoparticles using plant extracts for bactericidal purposes. Of those, Copper oxide nanoparticles (CuO) in particular show good ability to kill multi resistant bacteria, making them a potential competitor in new antibacterial treatments (Kim et al., 2012). (Velsankar et al. 2020) and (Andualem et al. 2020) used the extract of *Allium sativum* in synbiotic process to synthesis CuO nanoparticles and also the assessment of their antimicrobial effectiveness as well as antioxidant and anti larvicidal activities was performed. A study by (Bhumika K Sharma et al., 2018) investigated the eco-friendly synthesis of CuO nanoparticles utilizing *Azadirachta* indica leaf extract and assessed their antibacterial properties. These results suggested that CuO nanoparticles were capable of exhibiting potential antibacterial activity on being used against both Gram-positive and Gram-negative bacteria and may consequently be significant antimicrobial agents. Several recent studies by (Virk *et al.* 2023) by using *Aloe vera* extract synthesized CuO NPs which performed very important antibacterial activity to different pathogenic bacterial strains. Altogether these studies underscore the new trend in the synthesis of CuO NPs and their applications being introduced predominantly in the environmentally more friendly methods of using plant extracts as reducing agents which also showed pronounced antibacterial effects. Zinc oxide ZnO nanoparticles also have antibacterial properties and low toxicity which makes them desirable for biomedical (Sirelkhatim et al., 2015). Sirelkhatim and his collaborators discussed the antibacterial and low toxicity properties, suggesting their suitability for diverse biological applications. utilisation of plant extracts to the green synthesis of ZnO nanoparticles, discussing the antibacterial and low-toxicity properties in such a way suggesting their suitability for diverse biological applications. (Rao et al. 2021) also reported a study that discussed green synthesis of ZnO nanoparticles using *Camellia sinensis* extract as a reducing agent and its use in the antibacterial and antioxidant activities using ZnO nanoparticles. In addition, (Yadi et al., 2018) unveiling their excellent antibacterial activity and eventually confirming the formulation of ZnO nanoparticles with Ginkgo biloba leaf extract. Cumulatively, these results substantiate the eco-friendly phytosynthesis of ZnO nanoparticles coupled to efficient antibacterial and anti-oxidant performances due to the biomaterial. Silver oxide nanoparticles AgO NPs are in the top list of candidate as well with their excellent antibacterial effect and are already applied in plenty of health and consumer products (Temizel, et al., 2020). According to (Gupta et al. 2020) extract as reducing agent and their antimicrobial activity have been studied which provides very useful knowledge to the researchers and demonstrates superior antibacterial efficacy against different bacteria (Jyoti et al. 2020). Further, (Abdellatif et al. 202202) Investigates the Green Synthesis of Silver oxide Nanoparticles from Acanthopanax sessiliflorus Extract and Antibacterial and antioxidant Activities of These Products Other studies have shown that manganese oxide nanoparticles have antimicrobial properties, and because of these favorable physicochemical properties, their synthesis has been applied in various fields of science technology, including medicine and in biotechnology (Khan & Wahab, 2022). However, recent work has proven that MnO_2 NPs is a worthwhile candidate to act as a drug carrier for its biocompatibility and permeability through biological barriers. Another study by Cai et al., 2019, reported the potential applicability of manganese oxide nanoparticles as positive contrast agent for magnetic resonance imaging for diagnostic imaging purposes. In the nutshell, green synthesis of CuO, ZnO, AgO and MnO nanoparticles using *Moringa oleifera* leaf extracts is a highly effective and advanced method for the availability of novel antibacterial agents to combat antibacterial resistance, nowadays (Prasad & Elumalai, 2011).

These green-synthesized nanoparticles can be useful in antibacterial treatments and special applications. They can be potentially utilized for several biomedical applications like drug/gene carriers, wound healing and biosensing. Classified as nanomaterials, they are also considered environmental applications such as water purification and antimicrobial coatings, as interesting prospects for research and development. Further studies need to investigate the possible molecular interaction of these nanoparticles along with in-depth insights into the long-term stability and biocompatibility, or synthesis optimization to explore the potential for a larger production scale. Expanding the knowledge and applicability of 'green-synthesized' nanoparticle materials associated with antibiotics will ultimately help in fighting antibiotic resistance which in return is expected to improve public health. There could also be far-reaching economic implications from using these green synthesis methods, which might save money in production, as well as in regulatory controls on chemical synthesis.

In the current investigation, we propose a new environmental technique for the synthesis of metallic nanoparticles using *Moringa oleifera* extracts as a natural product providing rich bioactive compounds. A step by step procedure was adopted for the synthesis of bioactive compounds from *Moringa oleifera* leaves followed by the reduction of metal ions to nanoparticles. The nanoparticles were analyzed using X-ray diffraction (XRD) and transmission electron microscopy (TEM). The antidiarrheal activity of selected extracts was further assessed by applying crude extracts topically in vivo on castor oil induced diarrhea and in vitro with various antimicrobial activity tests like disk diffusion and minimum inhibitory concentration (MIC) tests against pathogenic bacterial strains. We speculate that nanoparticles derived from *Moringa oleifera* extracts possess strong potential antibacterial activity and are sufficiently nontoxic to be candidates for biomedical use. By addressing the synthetic and application challenges of metallic nanoparticles, this work introduces alternatives to conventional antibiotics for use in clinical settings.

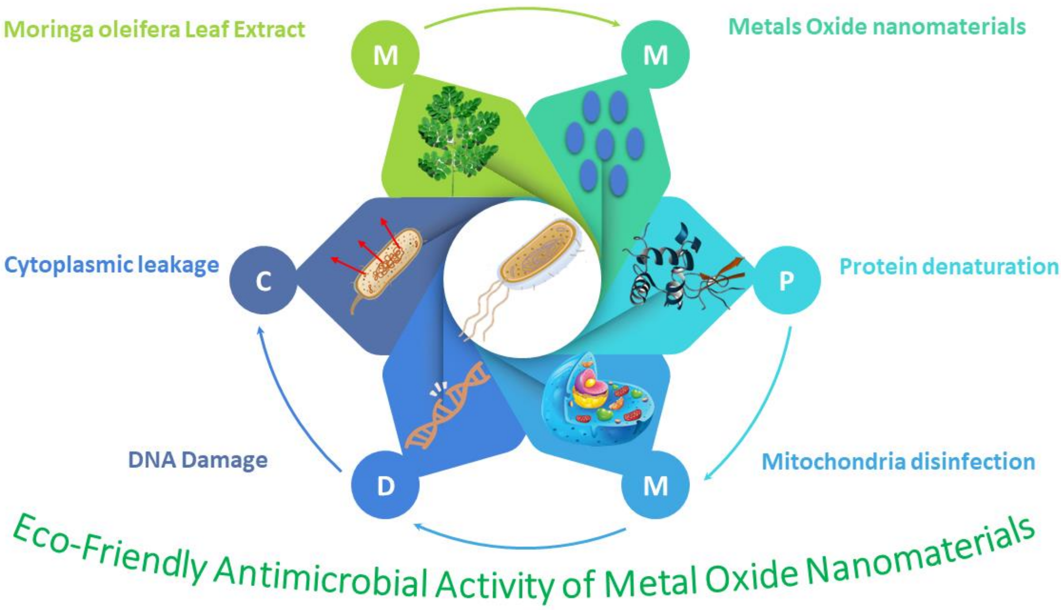

Fig. (1): Mechanism of the Eco-Friendly Antimicrobial Activity of Metal Oxide Nanomaterials

2. MATERIALS AND METHODS

2.1. Preparation of Moringa Leaf Extract

Moringa oleifera leaves were collected from the high plateaus of Algeria. The Moringa leaves, pre-treated by washing twice with demineralized water to remove all contaminating substances, were accurately weighed to (5 g) and finely ground. The resulting powder was placed in a beaker containing 100 mL of demineralized water and then put on a magnetic stirrer for 1 hour at a temperature of 80°C. After a period of maceration, the mixture was filtered using a paper filter to remove any remaining solid impurities, as illustrated in Fig. (2).

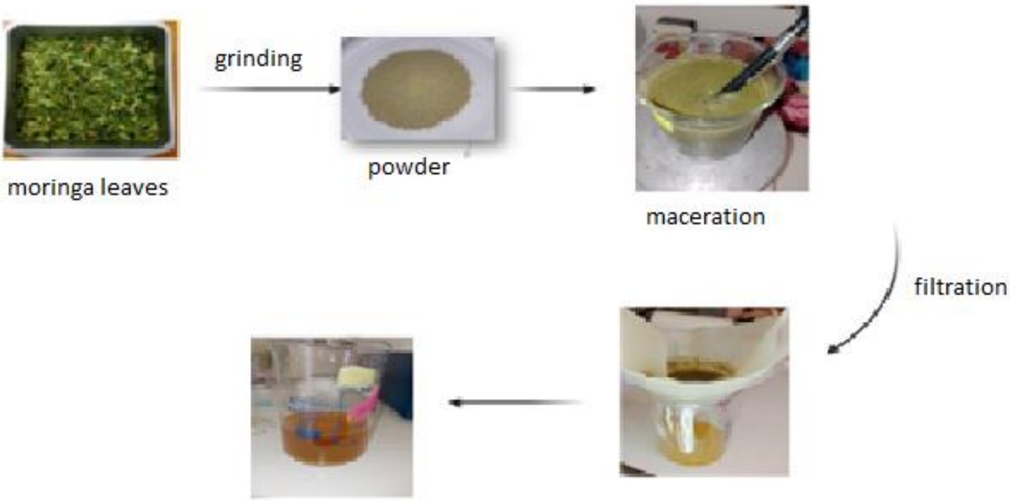

Fig. (2): Preparation of dried moringa leaf extract

2.2. Chemicals reagents and bacterial strains

All the chemical reagents used in this research were purchased from Sigma Chemical. Metal sulfates and nitrate ($CuSO_4$; $5H_2O$), ($AgNO_3$), (MnO_4S; H_2O) and ($Zn(NO_3)_2.6H_2O$) were of analytical grade and 98–100% pure, Mueller Hinton Agar medium was also received from Sigma Aldrich.

The three reference bacterial strains used are from the American Type Culture Collection (ATCC) and constitute excellent models for researching the antibacterial effects of different substances. These are: *Escherichia coli ATCC 25922 (G -), Staphylococcus aureus ATCC 25923 (G +) and Pseudomonas aeruginosa ATCC 27853*. These microorganisms are pathogenic and responsible for certain serious infectious diseases. They come from the microbiology laboratory of the Algerian University Hospital Center.

2.3. Synthesis of Metal Oxide Nanoparticles

Aqueous extracts of *Moringa oleifera* leaves were used in the precipitation process to synthesize metal oxide nanoparticles. The dried leaves were ground and macerated in demineralized water to prepare a 30 mL extract. This was then quickly added to the metal ions prepared beforehand from 10 mL of metallic salt solution each. The pH was adjusted to 10 using a 12 M sodium hydroxide (NaOH) solution and stirred constantly at a temperature of 80°C. These steps lead to the formation of metal oxide nanoparticles.

Characterization of Nanoparticles

The synthesized nanoparticles were characterized for their structure, size, and morphology. Crystallographic data from the X-ray powder diffraction of 2ϴ were collected between 10° and 80° with a scan rate of 1° min^{-1} at 25 C° on a Bruker D8 diffrac-tometer with a theta–theta geometry and a CuKα radiation (k=1.54056 A°), The crystallite size was determined by applying Scherrer's formula, which is as follows: $D = K\lambda/\beta\cos\Theta$, where K is the Scherrer's constant, typically taken to be 0.89, λ is the CuKa radiation's X-ray wavelength (k = 1. 1541 Å), β is the half-maximum line width in radians, and Θ is Bragg's diffraction angle. The X-ray spectra of the nanoparticles showed sharp diffraction peaks, confirming their crystalline structure. The features of the peaks were attributed to the specific crystalline phases of the metal oxide NPs. The average grain size of the Metal-Oxide/Moringa nanoparticles was measured from the micrographs obtained.

The functional groups correlated with the Metal-Oxide/Moringa nanoparticles were graphed by Fourier transform infrared spectroscopy (FTIR) and revealed the existence of characteristic absorption bands related to phenolic, amine, and alkene functional groups, indicating that molecules present in the *Moringa oleifera* extract function in reducing and stabilizing the nanoparticles. The Metal-oxide and *Moringa oleifera* leaf extract FTIR spectra were collected within the range of 200 cm^{-1} to 5000 cm^{-1}. SEM images demonstrated that the nanoparticles had a primarily spherical morphology with a uniform size distribution .

Antibacterial Activity Tests

The high antibacterial efficacy of the nanoparticles was evaluated by the agar diffusion method against two bacterial strains, *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923). Various concentrations of nanoparticles were tested to determine the minimum inhibitory concentration (MIC). Wells were made in agar plates and filled with the nanoparticles, following incubation, bacterial growth was observed to be inhibited very effectively.

3. RESULTS AND DISCUSSION

Interpretation of Characterization Data

The XRD technique was used to determine the crystal structure and particle size of the nanoparticles. X-Ray diffraction patterns of Metal-Oxide nanoparticles synthesized at 25 °C are presented in Figure 3. The range of the diffraction angle 2Θ was from 10 degree to 80 degree. The peaks observed in the spectra confirm the crystalline nature of the nanoparticles, which is an important factor for their physicochemical properties and antibacterial activity. The results show well-defined diffraction peaks which imply their crystalline and phase pattern in the Metal-Oxide crystalline lattice. The synthesized nanoparticles have an average crystal size of 44.70 nm, 27.04 nm, 37.11 nm, and 36.04 nm for ZnO, CuO, AgO, and MnO NPs, respectively, all exhibiting a consistent crystalline structure.

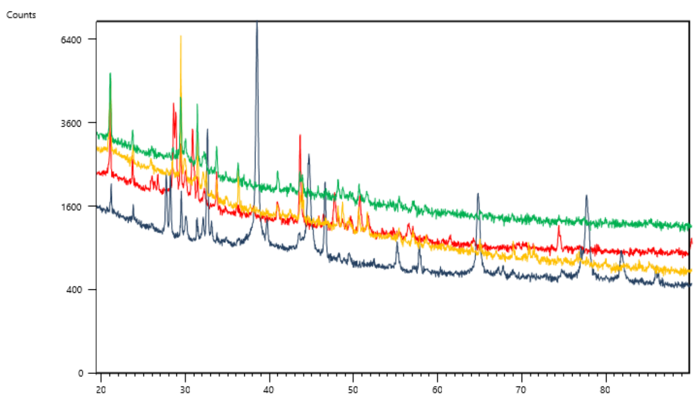

Fig. (3): XRD pattern of *M. oleifera* leaf extract assisted green synthesized Metal Oxide sample; Position [2Θ] ((CuO) Red, (ZnO) Yellow, (AgO) Blue and MnO Green).

The FTIR spectra illustrated in Figure 4, indicate that the phenolic and amine functional groups present in the *Moringa oleifera* extract play a key role in the reduction of metal ions to nanoparticles. These functional groups likely act as reducing and stabilizing agents, which is consistent with previous studies on nanoparticle biosynthesis. The spherical morphology and uniform size distribution confirmed in the SEM images are favorable characteristics that may influence the interaction of nanoparticles with bacterial membranes

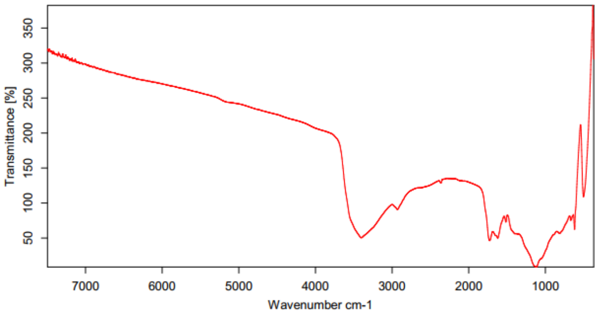

Fig. 4.a.

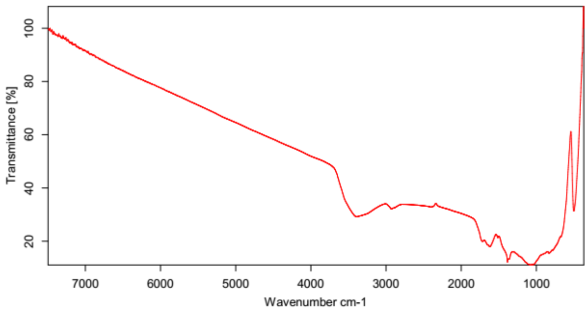

Fig. 4.b.

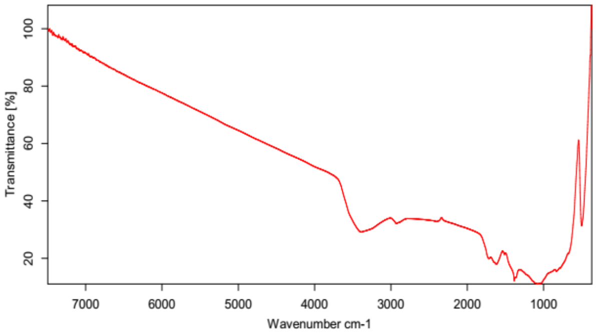

Fig. 4.c.

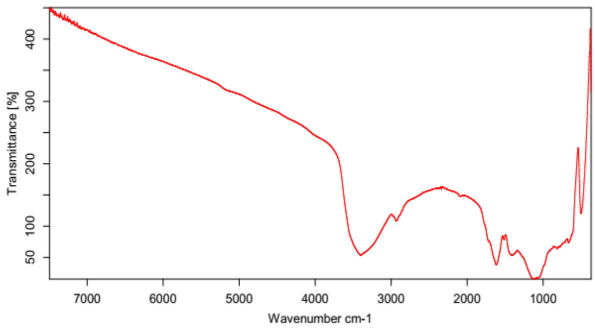

Fig. 4.d.

Fig. (4): FTIR spectrum of *M. oleifera* leaf extract assisted green synthesized **Fig. 4.a.** CuO nanoparticles, **Fig. 4.b.** AgO, **Fig. 4.c.** ZnO and **Fig. 4.d.** MnO.

SEM measurements were performed to examine the morphology of the prepared metal oxide samples. SEM images showing the shapes and configurations of various metal oxide nanoparticles provide essential information on the synthesis method and the reaction conditions used. CuO nanoparticles are able to aggregate or may present a more complex structure around 5 µm in diameter (Figure 5 a). The ZnO nanoparticles display an aggregated morphology with defined features, including crystalline planes, defects, or growth facets as shown in the SEM images (Figure 5 b). Silver oxide (AgO) nanoparticles occur in the form of tiny spherical, hexagonal, or irregularly shaped particles depending on the synthesis conditions (Figure 5 c). The MnO nanoparticles are further characterized by different morphologies such as spherical particles, granulates, or aggregates, or even more complicated structures of various sizes controlled by the processing conditions (Figure 5 d). The images reveal the morphology of the synthesized metal oxide nanoparticles with high clarity, providing valuable insight into the synthesis and properties of the resulting materials.

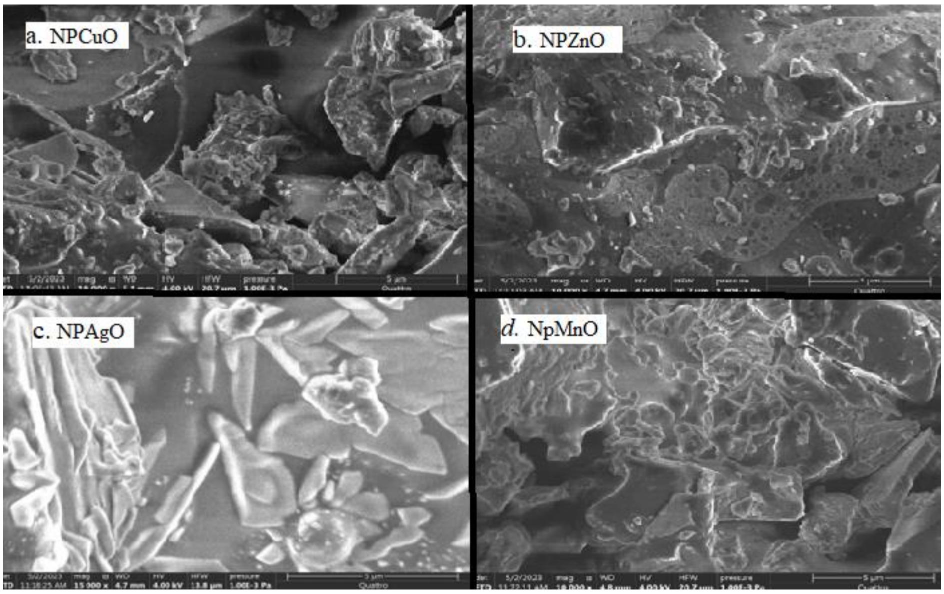

Fig. (5): SEM images of (a).NP CuO, (b) NP ZnO, (c).NP AgO and (d) MnO NPs synthesized using *M. oleifera* leaf extract.

Analysis of Antibacterial Activity

The antibacterial activity tests showed that all tested nanoparticles inhibited the growth of *E. coli* and S. aureus. The zone of inhibition was proportional to the concentration of the nanoparticles, with higher antibacterial activity observed for Ag and CuO nanoparticles. The MIC values confirmed that silver and copper nanoparticles possess the strongest antibacterial activity among the tested nanoparticles.

The increased antibacterial activity of Ag and CuO nanoparticles as shown in Figure 6 and Table 1 suggests that their size, shape, and chemical composition play a crucial role in their efficacy. The larger inhibition zones for these nanoparticles could be attributed to their higher oxidative potential and ability to induce cellular damage through oxidative stress. The correlation between the concentration of nanoparticles and the inhibition of bacterial growth suggests a dose-dependent effect, which is an important observation for the potential therapeutic application of these nanoparticles.

Considerations on Safety and Applicability

While our results are promising for the use of nanoparticles as antibacterial agents, further studies on cytotoxicity and biocompatibility are necessary. Green biosynthesis offers a safer and more environmentally friendly method for producing nanoparticles, but the viability on a large scale and the long-term environmental impacts need to be assessed.

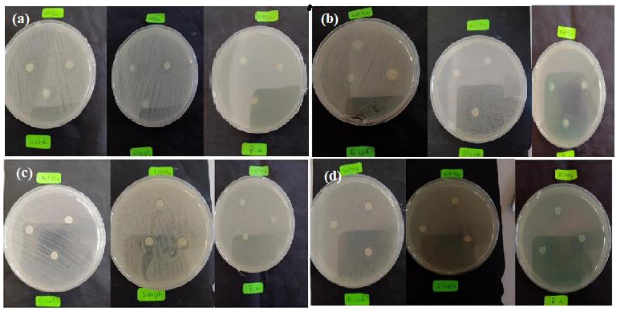

Fig. (6): Bactericidal inhibitory effects of hierarchical **(a)** CuO, **(b)** ZnO, **(c)** MnO and **(d)** (AgO) microspheres against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* respectively.

The results indicate that each metal oxide exhibits antibacterial activity for different bacteria, as Table 1 shows. The copper oxides (CuO) showed moderate to high efficiencies on *E. coli* (14.28-16.11 mm) and P. aeruginosa (9.42-9.55 mm); however, it has weak efficiency on *Staphylococcus aureus* (8.10-8.30 mm). The overall activity of zinc oxides (ZnO) is good, and the values of the inhibition zones in (mm) for E. coli, *Staphylococcus aureus*, and P. aeruginosa are between 10.68 to 12.90 mm, 10.23 to 12.51 mm, and 11.84 to 11.87 mm, respectively. Silver oxides (AgO) display moderate efficacy against *E. coli* (9.93-10.73 mm), but strong antimicrobial activity against *Staphylococcus aureus* (9.98-14.8 mm) and *Pseudomonas aeruginosa* (11.09-15.26 mm). In contrast, manganese oxides (MnO) also demonstrate potential inhibitory activity toward *E. coli* (from 7.83 to 8.28 mm) and are highly active towards *Staphylococcus aureus* (~11.77 mm) and *Pseudomonas aeruginosa* (from 10.78 to 12.72 mm). These observations imply that nanometric metal oxides display bactericidal activity that is specific to each bacterium, and that the choice of an oxide may be crucial for antibacterial efficiency.

Table 1: The table presents the results of antibacterial effectiveness tests of different nanometric metal oxides (NPs) against three bacteria: *Escherichia coli* (E. Coli), *Staphylococcus aureus* (Staphe), and *Pseudomonas aeruginosa* (P.a).

Bacterium / NPs	**E.Coli (mm)**	**Staphe (mm)**	**P.a (mm)**
NP CuO	16.11	8.30	9.42
	14.28	8.23	9.45
	---------	8.1	9.55
NP ZnO	12.90	12.51	11.87
	11.17	10.23	11.85
	10.68	---------	11.84
NPAgO	10.71	14.8	15.26
	10.73	9.98	13.94
	9.93	10.31	11.09
NP MnO	8.28	11.76	11.48
	7.83	11.77	10.78
	7.87	11.77	12.72

4. CONCLUSION

To sum up, the research on the study of metal oxides, characterizations, and antibacterial outcomes of nanoparticles synthesized through *Moringa oleifera* leaf extract have been found to be exciting. Using techniques including FTIR, XRD, and SEM, the synthesis of nanoparticles with unique physicochemical properties such as a crystalline nature and spherical structure has been successfully demonstrated. Phenolic and amine groups present in the *Moringa oleifera* extract were responsible for the reduction and stabilization of these nanoparticles.

Additionally, in vitro studies of antibacterial activity showed that nanoparticles AgONPs and CuO NPs had pronounced inhibitory effects against *E. coli* and *S. aureus*, suggesting their antimicrobial potential for application in nanobiotics. This activity is also dependent on size, shape, and composition, considering these parameters as pivotal factors for their antibacterial efficacy. The study offers one approach to addressing the issue of whether spherical nanoparticles of less than 5 nm in diameter are more toxic than other shapes and sizes of particles, regardless of composition, but it also points to the importance of conducting appropriate safety and environmental assessments of these nanoparticles when being considered for practical applications. The use of green synthesis in this study opens up a plethora of possibilities for an environmentally sustainable mode of nanoparticle production; however, the scalability and sustainability of the process in the long term need to be explored.

5. REFERENCES

1. Abdellatif, A. a. H., Alhathloul, S. S., Aljohani, A. S. M., Maswadeh, H., Abdallah, E. M., Musa, K. H., & Hamd, M. a. E. (2022). Green Synthesis of Silver Nanoparticles Incorporated Aromatherapies Utilized for Their Antioxidant and Antimicrobial Activities against Some Clinical Bacterial Isolates. Bioinorganic Chemistry and Applications/Bioinorganic Chemistry and Applications, 2022, 1–14. https://doi.org/10.1155/2022/2432758
2. Álvarez-Chimal, R., & Arenas-Alatorre, J. N. (2023). Green Synthesis of Nanoparticles: A Biological Approach. In IntechOpen eBooks. https://doi.org/10.5772/intechopen.1002203
3. Andualem, W. W., Sabir, F. K., Mohammed, E. T., Belay, H. H., & Gonfa, B. A. (2020). Synthesis of Copper Oxide Nanoparticles Using Plant Leaf Extract of Catha edulis and Its Antibacterial Activity. Journal of Nanotechnology, 2020, 1–10. https://doi.org/10.1155/2020/2932434
4. Cai, X., Zhu, Q., Zeng, Y., Zeng, Q., Chen, X., & Zhan, Y. (2019). <p>Manganese Oxide Nanoparticles As MRI Contrast Agents In Tumor Multimodal Imaging And Therapy</p> International Journal of Nanomedicine, Volume 14, 8321–8344. https://doi.org/10.2147/ijn.s218085
5. Gnanasangeetha, D., & Suresh, M. (2020). A Review on Green Synthesis of Metal and Metal Oxide Nanoparticles. Nature, Environment and Pollution Technology/Nature, Environment and Pollution Technology, 19(5(Supp)), 1789–1800. https://doi.org/10.46488/nept.2020.v19i05.002
6. De Kraker, M. E. A., Stewardson, A. J., & Harbarth, S. (2016). Will 10 Million People Die a Year due to Antimicrobial Resistance by 2050? PLoS Medicine, 13(11), e1002184. https://doi.org/10.1371/journal.pmed.1002184
7. Fifere, N., Airinei, A., Doroftei, F., Ardeleanu, T. S., Dobromir, M., Tîmpu, D., & Ursu, E. L. (2023). Phytomediated-Assisted Preparation of Cerium Oxide Nanoparticles Using Plant Extracts and Assessment of Their Structural and Optical Properties. International Journal of Molecular Sciences, 24(10), 8917. https://doi.org/10.3390/ijms24108917
8. Garadkar, K. M., Kadam, A. N., & Park, J. (2016). Microwave-Assisted Sol–Gel Synthesis Of Metal Oxide Nanomaterials. In Springer eBooks (pp. 1–22). https://doi.org/10.1007/978-3-319-19454-7_107-1
9. Gautam, S., Das, D. K., Kaur, J., Kumar, A., Ubaidullah, M., Hasan, M., Yadav, K. K., & Gupta, R. K. (2023). Transition metal-based nanoparticles as potential antimicrobial agents: recent advancements, mechanistic, challenges, and future prospects. Discover Nano, 18(1). https://doi.org/10.1186/s11671-023-03861-1
10. Gupta, A., Briffa, S. M., Swingler, S., Gibson, H., Kannappan, V., Adamus, G., Kowalczuk, M., Martin, C., & Radecka, I. (2020). Synthesis of Silver Nanoparticles Using Curcumin-Cyclodextrins Loaded into Bacterial Cellulose-Based Hydrogels for Wound Dressing Applications. Biomacromolecules, 21(5), 1802–1811. https://doi.org/10.1021/acs.biomac.9b01724
11. He, Z., Yang, H., Gu, Y., Xie, Y., Wu, J., Wu, C., Song, J., Zhao, M., Zong, D., Du, W., Qiao, J., Pang, Y., & Liu, Y. (2023). Green Synthesis of MOF-Mediated pH-Sensitive Nanomaterial AgNPs@ZIF-8 and Its Application in Improving the Antibacterial

Performance of AgNPs. International Journal of Nanomedicine, Volume 18, 4857–4870. https://doi.org/10.2147/ijn.s418308

12. Khan, F., & Wahab, R. (2022). Utilization of Solution Grown Manganese Oxide Nanocrystallite to Microstructure Against Bacteria's Inhibition. Journal of Inorganic and Organometallic Polymers and Materials, 32(5), 1650–1667. https://doi.org/10.1007/s10904-021-02206-8
13. Mahabadi, A. G., Mirzakhani, A., Azizi, A., Chavoshi, S., & Khaghani, S. (2021). Low-cost, simple and eco-friendly biosynthesis of CeO2-NPs using extract of Pelargonium hortorum for the photocatalytic and antioxidant applications. Research Square (Research Square). https://doi.org/10.21203/rs.3.rs-161201/v1
14. Manuja, A., Kumar, B., Kumar, R., Dharvi, Ghosh, M., Manuja, M., Brar, B., Pal, Y., Tripathi, B., & Prasad, M. (2021). Metal/metal oxide nanoparticles: Toxicity concerns associated with their physical state and remediation for biomedical applications. Toxicology Reports, 8, 1970–1978. https://doi.org/10.1016/j.toxrep.2021.11.020
15. Marchianò, V., Salvador, M., Moyano, A., Gutiérrez, G., Matos, M., Yáñez-Vilar, S., Piñeiro, Y., Rivas, J., Martínez-García, J. C., Peddis, D., Blanco-López, M. C., Rivas, M., Ditaranto, N., & Cioffi, N. (2021). Electrodecoration and Characterization of Superparamagnetic Iron Oxide Nanoparticles with Bioactive Synergistic Nanocopper: Magnetic Hyperthermia-Induced Ionic Release for Anti-Biofilm Action. Antibiotics, 10(2), 119. https://doi.org/10.3390/antibiotics10020119
16. Prasad, T., & Elumalai, E. (2011). Biofabrication of Ag nanoparticles using Moringa oleifera leaf extract and their antimicrobial activity. Asian Pacific Journal of Tropical Biomedicine/Asian Pacific Journal of Tropical Biomedicine, 1(6), 439–442. https://doi.org/10.1016/s2221-1691(11)60096-8
17. Rajak, K. K., Pahilani, P., Patel, H., Kikani, B., Desai, R., & Kumar, H. (2023). Green synthesis of silver nanoparticles using Curcuma longa flower extract and antibacterial activity. arXiv (Cornell University). https://doi.org/10.48550/arxiv.2304.04777
18. Rao, S. M., Kotteeswaran, S., & Visagamani, A. M. (2021). Green synthesis of zinc oxide nanoparticles from camellia sinensis: Organic dye degradation and antibacterial activity. Inorganic Chemistry Communications/Inorganic Chemistry Communications (Online), 134, 108956. https://doi.org/10.1016/j.inoche.2021.108956
19. Shahzadi, S., Zafar, N., & Sharif, R. (2018). Antibacterial Activity of Metallic Nanoparticles. In InTech eBooks. https://doi.org/10.5772/intechopen.72526
20. Singh, J., Dutta, T., Kim, K. H., Rawat, M., Samddar, P., & Kumar, P. (2018). 'Green' synthesis of metals and their oxide nanoparticles: applications for environmental remediation. Journal of Nanobiotechnology, 16(1). https://doi.org/10.1186/s12951-018-0408-4
21. Sirelkhatim, A., Mahmud, S., Seeni, A., Kaus, N. H. M., Ann, L. C., Bakhori, S. K. M., Hasan, H., & Mohamad, D. (2015). Review on Zinc Oxide Nanoparticles: Antibacterial Activity and Toxicity Mechanism. Nano-micro Letters, 7(3), 219–242. https://doi.org/10.1007/s40820-015-0040-x
22. Temizel-Sekeryan, S., & Hicks, A. L. (2020). Global environmental impacts of silver nanoparticle production methods supported by life cycle assessment. Resources, Conservation and Recycling, 156, 104676. https://doi.org/10.1016/j.resconrec.2019.104676
23. Velsankar, K., RM, A. K., R, P., V, M., & Sudhahar, S. (2020). Green synthesis of CuO nanoparticles via Allium sativum extract and its characterizations on antimicrobial, antioxidant, antilarvicidal activities. Journal of Environmental Chemical Engineering, 8(5), 104123. https://doi.org/10.1016/j.jece.2020.104123

24. Virk, P., Awad, M. A., Alsaif, S. S. a. L., Hendi, A. A., Elobeid, M., Ortashi, K., Qindeel, R., El-Khadragy, M. F., Yehia, H. M., El-Din, M. F. S., & Salama, H. A. (2023). Green synthesis of Moringa oleifera leaf nanoparticles and an assessment of their therapeutic potential. Journal of King Saud University. Science/Mağallaẗ Ğāmiʻaẗ Al-malik Saʻūd. al-ʻUlūm, 35(3), 102576. https://doi.org/10.1016/j.jksus.2023.102576
25. Yadi, M., Mostafavi, E., Saleh, B., Davaran, S., Aliyeva, I., Khalilov, R., Nikzamir, M., Nikzamir, N., Akbarzadeh, A., Panahi, Y., & Milani, M. (2018). Current developments in green synthesis of metallic nanoparticles using plant extracts: a review. Artificial Cells, Nanomedicine, and Biotechnology, 46(sup3), 336–343. https://doi.org/10.1080/21691401.2018.1492931