

Microbial activity of manganese nanoparticles prepared using *Citrus sinensis* leaf extract

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Abstract

The increasing demand for effective antimicrobial nanoparticles has led to the exploration of alternative approaches, including the use of plant extracts in the synthesis of metal nanoparticles. This study investigates the antimicrobial properties of orange leaf (*Citrus sinensis*) extract-mediated manganese nanoparticles. Green synthesis of manganese nanoparticles was done using the wet-impregnation method with orange leaf extracts and manganese nitrate. Characterization of MnNPs was done using Fourier transform infrared spectroscopy (FTIR), which confirmed the presence of stabilizing groups, including ester, ether, carbonyl, and hydroxyl groups, with peaks at 1100, 1200, 1350, 1500, 1800, and 3300 cm^{-1} . Scanning electron microscopy (SEM) revealed porous aggregates of MnNPs at 80 nanometers, while Electron dispersive spectroscopy confirmed the presence of a manganese atomic peak at 5.9 and 6.5 keV with a weight percentage of 91.31%, indicating successful synthesis. Antimicrobial analysis using the agar well diffusion method showed susceptibility of *Pseudomonas aeruginosa* and *Escherichia coli* (gram-negative bacteria) to MnNPs, with the highest activity observed against *Aspergillus niger* (fungi). In conclusion, orange leaf extract-mediated manganese nanoparticles exhibit significant antimicrobial activity, suggesting their potential as effective antimicrobial agents.

Keywords: Manganese; nanoparticles; *Citrus sinensis*; antimicrobial activity; green synthesis

1. Introduction

Nanoparticles have gained attention due to their valuable properties [1]. Their high surface area-to-volume ratio enables free and easy interaction and diffusion. research has focused on nanoparticles, discovering new areas and possibilities in nanotechnology [2-4]. Nanoparticle synthesis methods include physical, chemical, and green synthesis. However, chemical reduction lacks control over particle size and produces harmful and undesirable by-products [5]. Green synthesis, using plant extracts and microorganisms, offers a cheap efficient, and eco-friendly alternative [6, 7].

Nanoparticles have various applications, including disease diagnosis and management, drug delivery, and environmental remediation [8]. They are also used for agricultural purposes, cosmetics and medicinal purposes [9]. Among various metallic nanoparticles, silver nanoparticles have shown exceptional efficacy against bacteria, viruses, and microorganisms [10-12]. Extracts of plants contain various active ingredients, including alkaloids, phenols, proteins, terpenoids, quinines, amides, flavonoids, and alcohols [13-14]. These reducing active ingredients like flavonoids and phenols play a vital role in the green synthesis of metal nanoparticles (MNPs) by reducing metal cations and acting as stabilizers to prevent aggregation [13, 15].

The synthesis of MNPs using plant extracts involves three steps which are: reduction, nucleation, and termination [15, 16]. During reduction phase, reducing phytoactives reduce metal ions to zero-valent metal atoms through electron transfer. In the nucleation phase, atoms aggregate into nanometallic particles with various shapes. Finally, in termination phase phytoactive components with antioxidant properties stabilize the MNPs [13, 14].

Studies have shown the use of plant extracts in synthesizing MNPs. For example, Sajadi et al. used polyphenolic substances from *Silybum marianum* seed extracts to reduce copper ions to CuNPs [17]. Similarly, Yu et al. utilized nanocellulose to synthesize silver nanoparticles, where positively charged, silver ions were attracted to negatively charged carboxyl and hydroxyl groups [18]. Jigyasa and Rajput used polyphenols in the synthesis of silver nanoparticles proposing that curcumin reduced silver ions through carbonyl or phenolic hydroxyl groups [19].

Extracts of plants possess both reducing and antioxidant properties, enabling them to serve as reducing agents and stabilizers in metal nanoparticles synthesis [20, 21]. This study investigates the antimicrobial properties of orange leaf (*Citrus sinensis*) extract mediated Manganese nanoparticles.

2. Materials and Methods

2.1 Sample collection

The sample collection was conducted at the College of Health Sciences, Niger Delta University, Bayelsa, Nigeria. The exact location was determined using a satellite map, with the coordinates 4.968887° N latitude and 6.097664° E longitude. Clean, sterile equipment, including gloves, scissors, and collection bags, was used to collect the samples.

2.2 Preparation of the plant extract

Plant leaves (40 g) were washed with tap water, followed by distilled water to remove the dirt and other impurities, and dried well. The leaves were sliced into very tiny parts, ground, and then boiled for 60 min at 70 °C with 200 mL of distilled water in a 500 mL round bottle flask. After the mixture was allowed to reach room temperature, the resultant mixture was filtered with the help of a strainer to eradicate any solid content and finally filtered through Whatman filter paper. The filtrate was stored at 5 °C in a refrigerator for the synthesis of manganese nanoparticles.

2.3 Synthesis of manganese nanoparticles

Exactly, 100 mL of 1 mol manganese nitrate $Mn(NO_3)_2$ solution was prepared in distilled water in a 500 mL beaker, and 50 mL of orange plant leaves extract was slowly mixed with continuous stirring at 70–80 °C on a magnetic stirrer. After 4 hours of vigorous stirring, mixture was filtered upon settling. The collected precipitate of MnNPs was washed 4–5 times with distilled water and dried on a watch glass transferred to a crucible, and oven dried at 60 °C for 7 hours before further use.

2.4 Characterization of synthesized manganese nanoparticles

The synthesized manganese nanoparticles were characterized using Fourier transform infrared spectroscopy (FTIR) (PerkinElmer Spectrum 100) to determine the functional groups present. The morphology and elemental analysis of the synthesized MnNPs was examined using advanced scanning electron microscope (SEM-EDX) (JEOL JSM-6390) at an accelerating voltage of 15 kV.

2.5 Antimicrobial assay

Antimicrobial analysis was carried out using Muller-Hinton Agar plate. Seven pathogenic organisms which include five bacteria: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Escherichia coli*, and *Ralstonia Solanacearum* with two fungi *Penicillium notatum*, and *Aspergillus niger* were used in the antimicrobial assay. The test microorganism was inoculated onto the agar plates using a sterile loop after which sterile well cutters were used to create wells in the agar plates. The test substance was added to the wells at varied concentration of 25, 50 and 100 mg/L using a micro pipette.

The plates were incubated at a controlled temperature of 37 °C for 24 hours. The zone of inhibition (Z.I.) was measured using a ruler.

3. Results and Discussion

3.1 Characteristics of synthesized manganese nanoparticles

Manganese nanoparticles were synthesized (product shown in Figure 1) and characterized using SEM and FTIR. Figure 2 shows the morphology of the Mn-nanoparticle at 80 nm, showing the formation of uniformly distributed quasi-spherical porous shape. Figure 3 shows the Fourier transform infrared spectra of Mn-nanoparticles with characteristic peaks around 1100, 1200, 1350, 1500, 1800 and 3300 cm^{-1} assigned to C-O, C=O, C-C and O-H groups absorption bands as the possible stabilizing and capping groups from the *Citrus sinensis* leaf extracts. Figure 4 shows the electron dispersive X-ray of the synthesized MnNPs with a manganese atomic peak of 91.31 weight percentage and energy of 5.9 and 6.5 Kev, indicating successful MnNPs synthesis.



Figure 1: Synthesized manganese nanoparticles

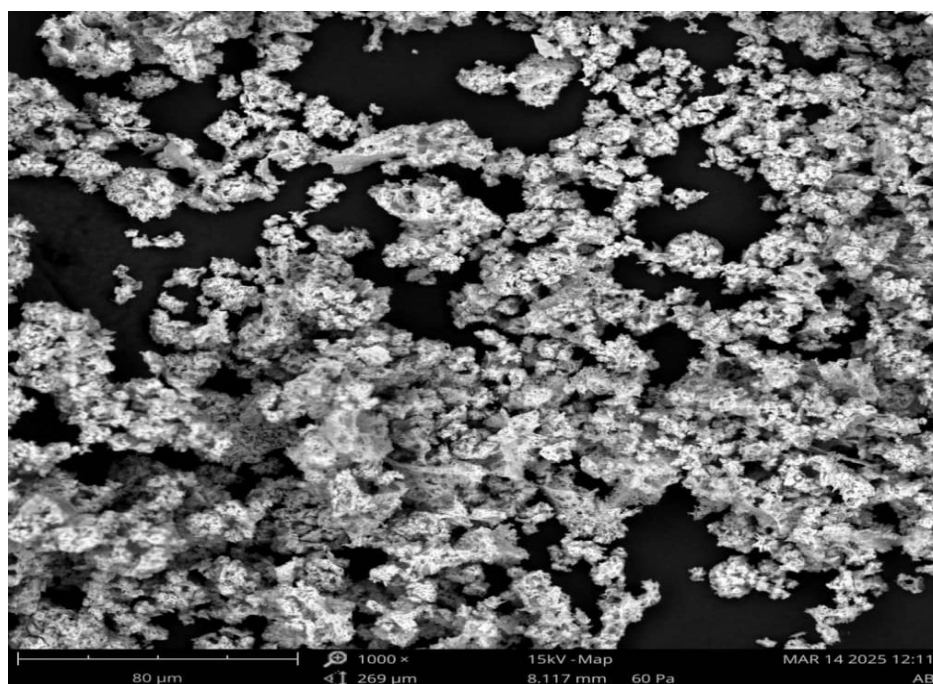


Figure 2: Surface morphology of the synthesized MnNPs obtained via SEM

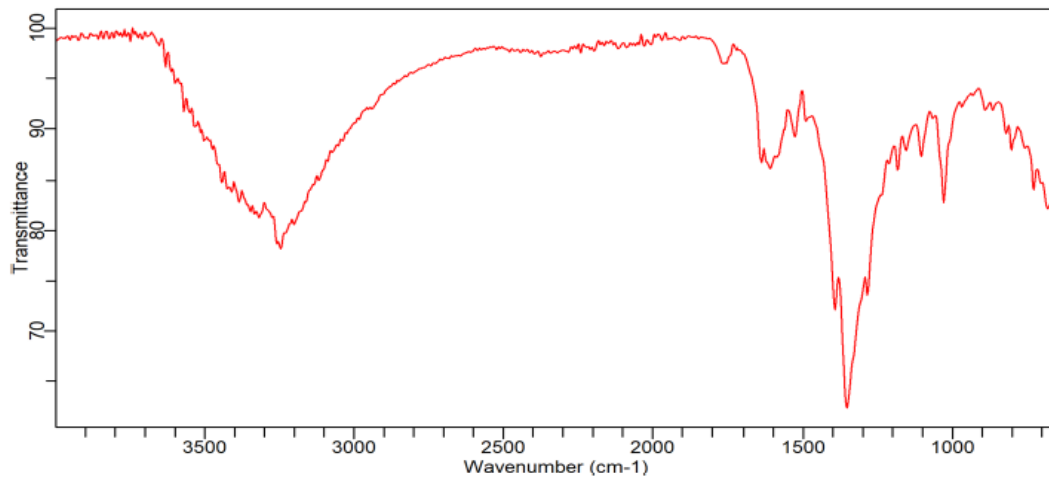


Figure 3: FTIR spectra of the synthesized manganese nanoparticles

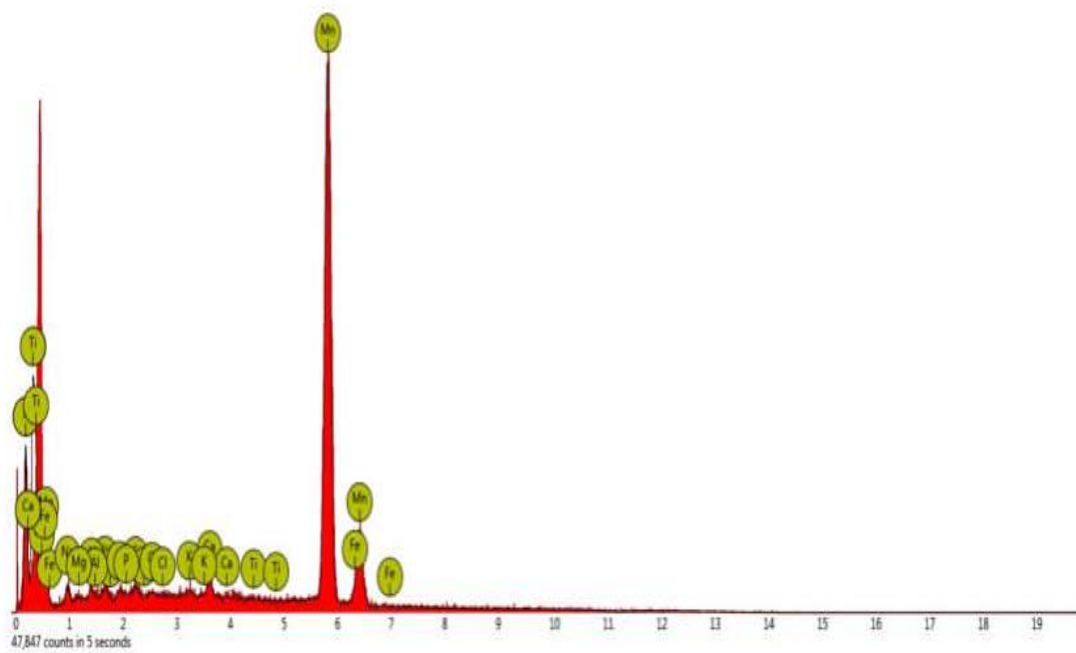


Figure 4: EDX spectra of the synthesized manganese nanoparticles

3.2 Antimicrobial Studies

Citrus sinensis manganese nanoparticles showed concentration-dependent zone of inhibition against all the tested microorganisms at varied concentrations when 100, 50 and 25 mg/L of the nanoparticles were used. The gram-negative bacteria, *Pseudomonas aeruginosa* and *Escherichia coli* were the most susceptible organisms while the highest activity was shown against *Aspergillus niger* for fungi (Table 1). This could as a result of gram-negative pathogens mostly containing only a single peptidoglycan coat, hence making the migration of manganese (Mn) ions into the cytoplasm easy, resulting in cytolysis [20].

Table 1: Zone of inhibition of the synthesized MnNPs against strains of microorganisms

Organism	Zone of inhibition at various MnNP concentration		
	100 mg/L	50 mg/L	25 mg/L
<i>Staphylococcus aureus</i>	21	18	16
<i>Escherichia coli</i>	22	18	16
<i>Pseudomonas aeruginosa</i>	22	18	16
<i>Klebsiella pneumonia</i>	18	16	14
<i>Aspergillus niger</i>	18	16	14
<i>Penicillium notatum</i>	16	14	10
<i>Ralstonia solanacearum</i>	18	16	14

4. Conclusion

The study demonstrates the potential of using green synthesis methods to produce antimicrobial nanoparticles. The results show that manganese nanoparticles synthesized using *Citrus sinensis* leaf extract exhibit promising antimicrobial activity, particularly against gram-negative bacteria.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

Chinaemerem Ebenezer Iheme: Conceptualization, Methodology, Investigation, Data Analysis, Writing - Original Draft; **Ebierin Harriet Boroh :** Methodology, Investigation, Review

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