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Antibacterial Activity of *Piper betle* Leaf Extract Mediated MnO_2 Nanoparticles

P. Subramanian [a] and S. Alwin David [b*]

[a] Department of Educational Planning and Administration, Tamil Nadu Teachers Education University, Chennai – 97, Tamil Nadu, India.
[b] PG & Research Department of Chemistry, V. O. Chidambaram College, Tuticorin – 628008 affiliated to Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli - 627 012, Tamil Nadu, India.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The biogenesis of manganese dioxide nanoparticles using *Piper betle* leaf extract is explained in this work as a simple, environment friendly and effective process. Using *Piper betle* leaf extract as a reducing agent, potassium permanganate was reduced to synthesize the MnO_2 NPs. The contribution of the biomolecules in the *Piper betle* leaf extract to the production of MnO_2 NPs was revealed by Fourier-transform infrared spectra. The biosynthesized MnO_2 NPs' UV–visible spectra showed absorption peaks around 271 nm, which is the MnO_2 NPs' absorption maxima. According to the scanning electron microscopy study, the 70–80 nm-sized biosynthesized MnO_2 NPs exhibit an irregular form. The presence of Mn and O in the MnO_2 NPs was verified by EDAX. MnO_2 NPs were tested for their antibacterial properties against *Bacillus subtilis, Bacillus cereus,* and *Bacillus amyloliquefaciens*. The antibacterial activity of the biosynthesized MnO_2 NPs is mild.

**Corresponding author: Email: alwindavid1986@gmail.com;*

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Keywords: MnO_2 NPs; biosynthesis; piper betle; antibacterial activity.

1. INTRODUCTION

Many in-depth researchers have examined metal oxides because of their affordable price, varying oxidation states, superior physical and chemical characteristics, and numerous industrial uses.

When it comes to inorganic materials, manganese oxide stands out among the other metal oxides because of its abundance, affordability, environmental friendliness, multiple oxidation states, rich compositional variations, larger surface area, ease of preparation using readily available solvents, and remarkable physical and chemical properties.

“Manganese oxide nanoparticles are becoming more and more important due to their wide range of applications, including the immobilization of proteins, lithium-air batteries, lithium-ion batteries, sensitive biosensors, electrochemical capacitors, biomimetic catalysts, formaldehyde removal analysis, adsorption, gas sensor, lithium-sulfur batteries, photocatalytic dye degradation, high-performance supercapacitors, wastewater treatment, energy storage, p-nitrophenol sensor, mineral supplementation in animal feed, raw materials for fertilizer, sensors molecular sieve, rechargeable batteries, magnetic materials, optoelectronic devices, chemical sensing devices, microelectronics, chemical sensing devices, catalysis, ion exchange, and antimicrobial activity” [1].

Chemical synthesis necessitates expensive, hazardous, combustible, and caustic substances that are frequently bad for the environment and people's health. This is why the development of a green method for nanoparticle manufacturing has received interest recently. In addition to being less harmful to the environment than other physiochemical techniques, green synthesis yields nanoparticles with superior size and morphology that are also inexpensive, non-toxic, biocompatible, and free of contamination.

Plant extracts, bacteria, yeast, fungi, actinomycetes, lichen, or algae have all been suggested as advantageous substitutes for physiochemical methods in the green manufacture of metal oxide nanoparticle.

'The synthesis of nanoparticles using plant extracts is less expensive than microbial synthesis, as the latter involves higher costs associated with producing microorganisms. Because plant extracts include a variety of bioactive chemicals that can be used as stabilizing and reducing agents, plant extract mediated nanoparticle synthesis is thought to be more effective than microbe mediated synthesis. It has been discovered that thousands of plant extracts can reduce metal salts to the matching metal oxides.

Manganese oxide nanoparticles have been synthesized from plant extracts, including those of *Adhatoda vasica Nees, Aegle marmelos, Ananas comosus, Dittrichia graveolens (L.), Gardenia resinifera, Kalopanax pictus, lemon, Matricaria chamomilla L., Phyllanthus amarus, Sapindus mukoross, Momordica charantia,* and *Yucca gloriosa*, according to numerous researchers. According to a thorough review of the literature, this is the first publication on the synthesis of MnO_2 NPs utilizing *Piper betle* leaf extract [2-20].

2. MATERIALS AND METHODS

2.1 Chemicals Used

Sigma Aldrich supplied the potassium permanganate needed for the production of MnO_2 NPs. Fresh and healthy *Piper betle* leaves were gathered at Thoothukudi, Tamilnadu, India.

2.2 Preparation of *Piper betle* Leaf Extract

Ten grams of fresh *Piper betle* leaves were carefully washed under running tap water and then dried with distilled water to get rid of any remaining dust. In a round-bottom flask with a condenser, 100 mL of distilled water was heated with finely crushed *Piper betle* leaves for one hour at 100°C. Whatman No. 41 filter paper was employed to filter the leaf extract.

2.3 Biosynthesis of MnO_2 NPs

Potassium permanganate ($KMnO_4$) and *Piper betle* leaf extract were utilized as the precursor salt and reducing agent, respectively, in the production of MnO_2 NPs.

In a round-bottom flask with a condenser, 75 mL of 0.1M potassium permanganate solution was mixed with 25 mL of *Piper betle* leaf extract. For two hours, this combination was

heated at 100°C. The synthesized MnO_2 NPs were then filtered and allowed to dry for a whole night at 60°C in an oven.

2.4 Antimicrobial Study

Using the disc well-diffusion technique on an agar plate, the antibacterial activity of the samples was assessed against the test bacterium. One milligram per milliliter of the material was placed into each sterile disc, and the plates were incubated at 37°C for a whole day. The bacteria cultures were briefly incubated and swabbed uniformly on each individual plate using sterile cotton swabs on the Muller Hinton Agar. The existence of inhibition zones surrounding the discs, measured in millimeters, served as proof of the inhibitory effect.

Testing was done to determine the antibacterial activity of MnO_2 NPs biosynthesized from *Piper betle* leaf extract against *Bacillus cereus, Bacillus amyloliquefaciens, and Bacillus subtilis*.

2.5 Characterization

The JascoV-600 spectrophotometer was used to record the UV-visible spectra of the MnO2 NPs and the extract from *Piper betle* leaves. Thermo Scientific Nicolet iS5 FTIR spectrometer was used to measure FTIR. A TESCAN MIRA3 XMU device was used to conduct energy dispersive X-ray analysis (EDAX) and scanning electron microscopy (SEM).

3. RESULTS AND DISCUSSION

3.1 UV-Vis Spectroscopy

One of the most effective methods for describing metal oxide nanoparticles is UV-visible spectroscopy, which also offers details on the optical characteristics of the particles.

The UV-visible spectrum of green synthesized 0.1M MnO_2 NPs is displayed in Fig. 1. One absorption peak, matching to the absorption maxima of manganese oxide nanoparticles, can be seen in the spectrum at approximately 271 nm.

3.2 FTIR Analysis

The FTIR spectrum of MnO_2 NPs (Fig. 2) shows major peaks at 555, 921, 1384, 1498, 1633 and 3404cm^{-1}.

The broad band at 3404cm^{-1} is due to the O–H stretching of alcohols or phenols. The band at 1633 cm^{-1} region is characteristic of C= O stretching of aldehydes or ketones. The band at 1498cm^{-1} is characteristic of N–O asymmetric stretching of nitro compounds. The spectrum clearly shows band for C-H bending vibration at 1384 cm^{-1}. These results suggest that many biologically active phytomolecules are left adsorbed on the surface of the MnO_2 NPs.

The lower absorption bands at about 555cm^{-1} and 921cm^{-1} are attributed to the O–Mn–O vibrational mode as reported for many MnO_2 NPs.

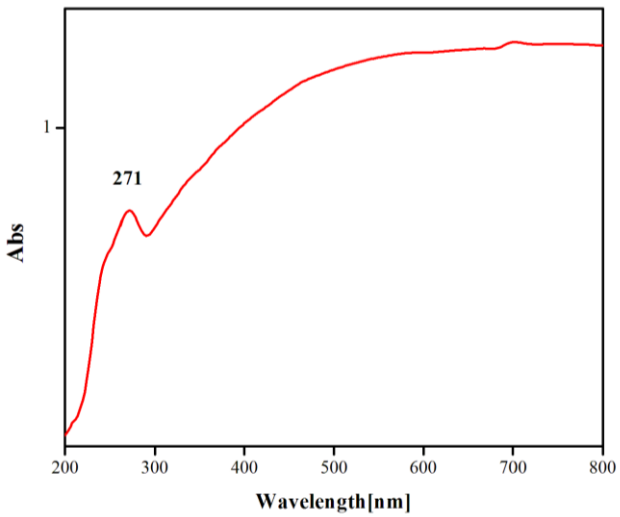

Fig. 1. UV-Visible spectrum of MnO_2 NPs

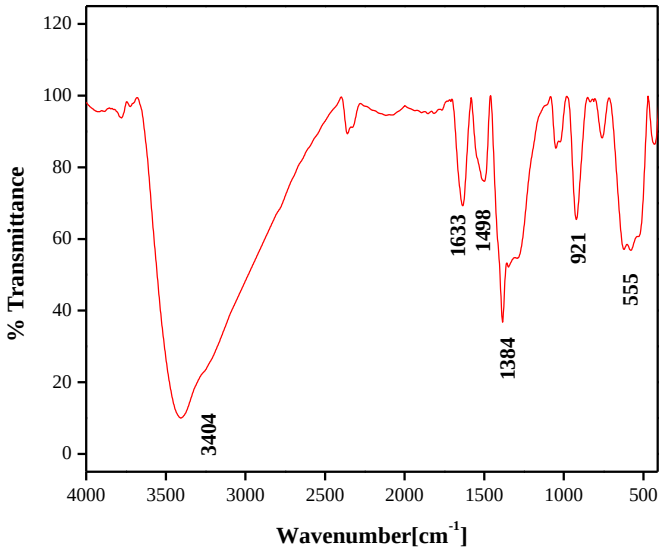

Fig. 2. FTIR spectrum of MnO_2 NPs

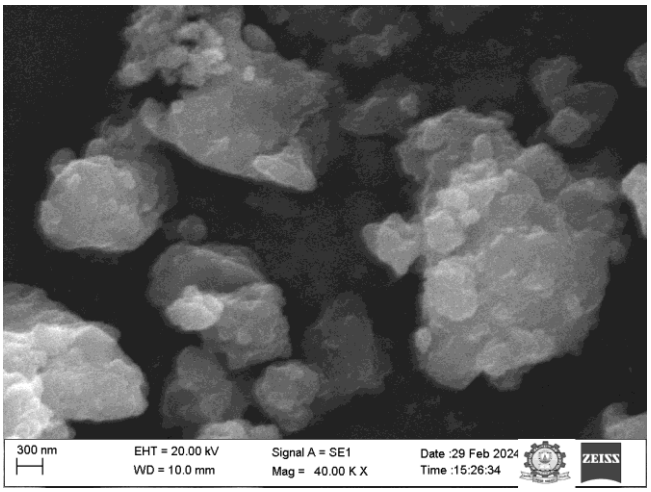

Fig. 3. SEM image of MnO_2 NPs in 300 nm scale

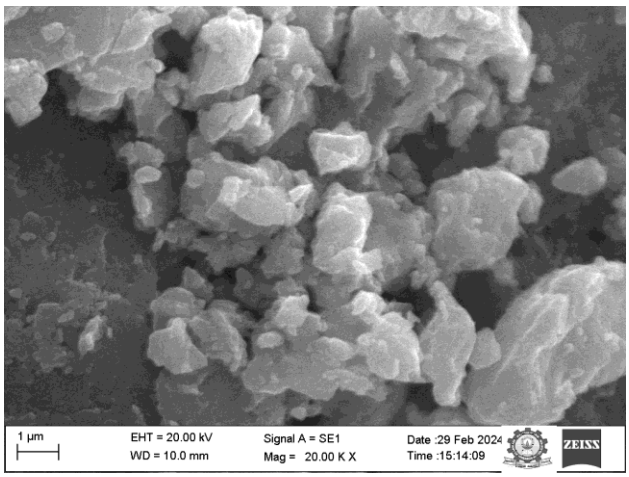

Fig. 4. SEM image of MnO_2 NPs in 1 µm scale

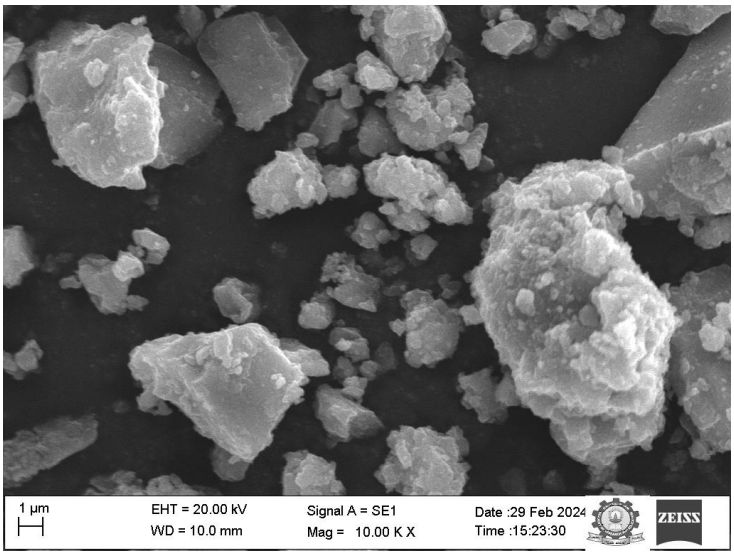

Fig. 5. SEM image of MnO_2 NPs in 1 µm scale

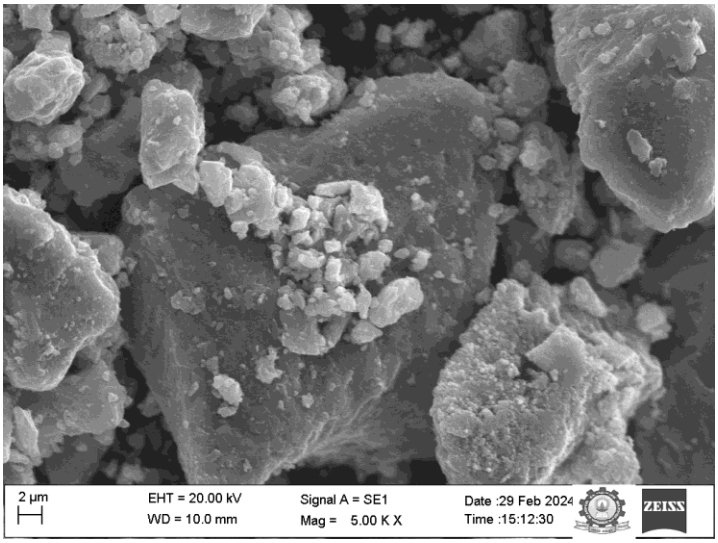

Fig. 6. SEM image of MnO_2 NPs in 2 µm scale

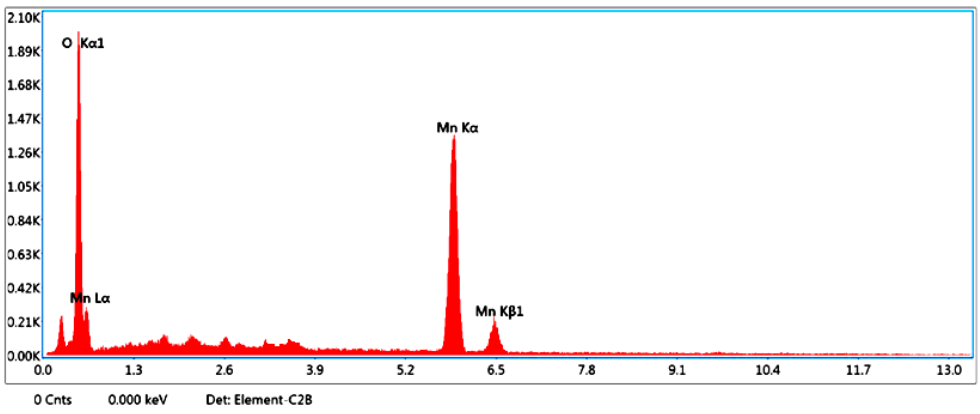

Fig. 7. EDAX spectrum of MnO_2 NPs

Table 1. EDAX data of MnO_2 NPs

Element	Weight %	Atomic %
Mn	74.4	45.9
O	25.6	54.1
TOTAL	100.00	100.00

Table 2. Antibacterial activity data of MnO_2 NPs

Bacteria	Inhibition zone in mm	
	Concentration 1mg/ml	
	Ampicillin	MnO_2 NPs
Bacillus amyloliquefaciens	20	11
Bacillus cereus	20	7
Bacillus subtilis	22	12

3.3 Scanning Electron Microscopy (SEM)

The surface shape and approximate size of the MnO_2 NPs are revealed by the SEM. The SEM pictures (Figs. 3–6) demonstrate the uneven morphology of MnO_2 NPs. The biosynthesized MnO_2 NPs have a size between 70 and 80 nm.

3.4 Energy Dispersive X - ray Analysis (EDAX)

The atomic contributions of the components in the nanoparticles were ascertained using energy dispersive X-ray analysis, or EDAX. The weight (%) of Mn and O in the EDAX of MnO_2 NPs (Fig. 7) are 74.4 and 25.6, respectively. For Mn and O, the atomic (%) is 45.9 and 54.1, respectively.

3.5 Antimicrobial Activity

The antimicrobial efficacy of biosynthesized MnO_2 NPs against *Bacillus subtilis, Bacillus cereus,* and *Bacillus amyloliquefaciens* has been investigated. *Bacillus amyloliquefaciens, Bacillus cereus,* and *Bacillus subtilis* are the three microorganisms against which biosynthesised MnO_2 NPs exhibit moderate antibacterial action.

4. CONCLUSION

Piper betle leaf extract was used as a reducing agent to successfully produce MnO_2 NPs from potassium permanganate precursor. The synthesis of MnO_2 NPs is confirmed by absorption bands in UV-visible spectra at 271 nm. FT-IR spectra show bands at 555 cm^{-1}, which support the existence of the O-Mn-O bond. SEM reveals that MnO_2 NPs have a varied size range of 70 to 80 nm and an irregular shape. The presence of oxygen and manganese in the MnO_2 NPs is confirmed by EDAX. When it comes to *Bacillus subtilis, Bacillus cereus*, and *Bacillus amyloliquefaciens,* MnO_2 NPs show moderate antibacterial activity.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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