Biogenic MnO_2 nanoparticles for antibacterial dental application

N.A. Abdullah[1], *Alaa* Mohsin Naser[2*], *Amaal* Al-Nuaimy[3], and *Munaf* M. Naji[4]

[1]Department of Physics, College of Science, University of Basrah, Iraq
[2]Al-Iraqia University, Baghdad, Iraq
[3]Department of Radiology, College of Dentistry, Aliraqia University, Baghdad, Iraq
[4]Department of Clinical and Laboratory Sciences, College of Pharmacy, Kufa University, Najaf, Iraq

Abstract. This study explores the biological synthesis and antibacterial activity of manganese dioxide (MnO_2) nanoparticles for potential use in dental infection diagnostics and control. MnO_2 nanoparticles were synthesized using an eco-friendly biogenic method based on leek (*Allium ampeloprasum*) leaf extract, serving as a natural reducing and stabilizing agent. Structural and morphological characterization confirmed the rhombohedral crystalline phase and compact nanostructure of the particles through X-ray diffraction and field-emission scanning electron microscopy analyses. Bacterial isolates from dental patients were identified and tested for susceptibility to MnO_2 nanoparticles. The nanoparticles exhibited strong antibacterial effects, with higher concentrations resulting in a significant increase in bacterial cell death. These findings highlight the biological potential of green-synthesized MnO_2 nanoparticles as sustainable bio-nanomaterials for diagnostic and antimicrobial applications in oral health management.

1 Introduction

Nanoparticles are characterized by the smallest particles, ranging in size from 1 to 100 nanometers in at least one dimension. Advances in biotechnology and nanotechnology are facilitating investigation in the domain of nanoparticle synthesis. Nanoparticles are widely used in cancer, allergies, diabetes, inflammation, and infection. They also have multiple applications in cosmetics, biomedical devices, medicine, renewable energy, and environmental remediation [1]. Over the past two decades, metal oxide nanoparticles have received increasing attention due to their unique and exceptional physical and chemical properties [2, 3]. Among these innovative technologies, green synthesis has emerged to enhance environmental sustainability by reducing and disposing of harmful compounds and hazardous waste [4]. Recent advances in nanotechnology have shown great potential in anti-caries materials. The current research demonstrates that nanoparticles of inorganic material can diminish biofilm formation and facilitate the remineralization of carious lesions, therefore averting dental caries. Metallic nanoparticles facilitate biomineralization, hence enhancing the restoration of minerals in demineralized tooth tissue. Moreover, owing to their

* Corresponding author: alaa.m.naser@aliraqia.edu.iq

ionic equilibrium in saliva, metallic nanoparticles can resolve issues in various oral situations [5]. Metallic nanoparticles and also metal oxides have been integrated into dental materials to inhibit caries development and improve the mechanical qualities of these materials. Moreover, the scalable production of inorganic nanoparticles is straightforward. Inorganic nanoparticles can function as direct antibacterial agents, thereby mitigating dental caries, one of the most prevalent bacterial-related oral disorders [6]. Manganese dioxide is an important nanoparticle commonly used due to its unique optical, thermal, mechanical, chemical, and electrical capabilities. Green synthesis of nanoparticles utilizes an eco-friendly approach, wherein biological components serve as reduction and cap agents, eliminating the necessity for large energy inputs or hazardous chemicals. This study involved the environmentally sustainable synthesis of manganese dioxide nanoparticles. This green technology is significant because it represents an environmentally friendly approach that utilizes natural biological resources, avoiding hazardous chemicals, to produce diverse nanoparticles. This green technology uses leek extract as a reducing and coating agent for nanoparticles to investigate the influence of nanoparticle synthesis on the antibacterial activity exhibited in well diffusion assays and the physical characteristics of MnO2 nanoparticles [7, 8].

2 Materials and methods

2.1 Preparation of the Leek leaf extract

To create an aqueous extract of Leek leaves, a quantity of 10 grams of the leaves was measured and combined with 100 ml of distilled water. The mixture was subsequently homogenized using a magnetic stirrer at a temperature of 35 °C for of 1 hour, until the solution transitioned from a watery consistency to a green hue. The ultimate outcome was achieved by passing the solution through a filter paper and subjecting it to centrifugation at a speed of 4000 revolutions per minute for 10 minutes. The obtained solution was subsequently preserved in a refrigerator at a temperature of 4°C until it was utilized.

2.2 Green Synthesis of MnO2 NPs

Preparing manganese dioxide nanoparticles was done by adding 10 grams of the extract to 1 gram (0.14Mm) of manganese sulfate monohydrate (MnSO4.H2O). The mixture was placed on a magnetic stirrer at 30 °C for 2 hr. The filtrate was separated from the precipitate by centrifuging it at 5000 rpm for 10 minutes. The sample was washed three times with deionized water and dried at (400,300,200) °C for 2-3 hours. Finally, the manganese dioxide nanoparticles were ground to obtain a blackish-brown-colored powder as shown in Figure 1.

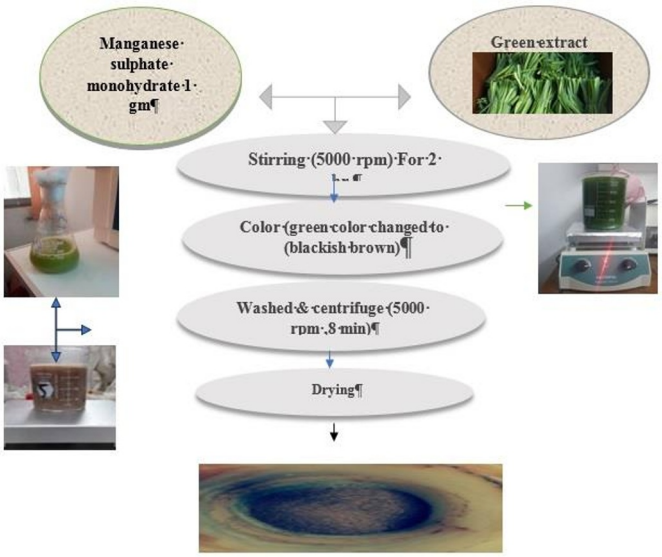

Fig.1. MnO2 -NPs powder by biosynthesis

2.3 Collection of study samples and Radiographic (OPG) images

Ten samples of both sexes were randomly selected from 8 male and 2 female patients who had caries and oral infections at the dental clinics at the Iraqi University, as shown in Table 1.

Table 1. Collecting Study Samples

Age	Sex	Oral hygiene	Site of swab
7	Male	Fair	Badly caries upper right A B C
4	Male	Fair	Badly caries upper left D E
45	Female	Poor	Buccal mucosa
21	Male	Good	Tongue
47	Male	Poor	Buccal mucosa
47	Male	Poor	Palate mucosa
58	Male	Poor	Periodontitis
39	Male	Poor	Periodontitis
20	Female	Good	Tongue
40	Male	Poor	Periodontitis

Three elderly patients presented with increased mobility of the lower anterior teeth, caries in the upper molars, and mild discomfort. Clinical examination revealed signs of periodontitis. Panoramic radiographs, as shown in Figure (2), showed moderate to severe horizontal bone loss in the lower anterior region. The condition was characterized as chronic generalized periodontitis. Oral swab samples were collected from the gingival margins and

periodontal pockets in the affected area. These samples were preserved and sent for microbiological analysis to determine the pathogenic bacterial load and inflammatory response.

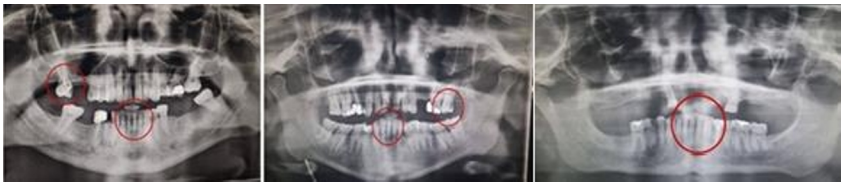

Fig.2. OPG X-ray patients' pictures

2.4. Identification via the Vitek II system

The technique was utilized according to the recommendations of the manufacturing business (bioMérieux, France) by inoculating bacteria onto a blood agar plate, followed by incubation at 37 ºC for 24 to 72 hours. A bacterial suspension was created. One to 3 colonies were transferred to a test tube with 3 ml of normal saline, adjusting the turbidity to a McFarland standard of 0.5.

2.5. Formulation of Antibacterial Agents

An initial solution of pure MnO2NPs was produced in 5 ml tubes to the specified concentration and subsequently passed through a 0.22 Millipore filter. Serial two-fold dilutions of the stock solution were executed utilizing Mueller-Hinton broth (MHB) in a 96-well plate. 100 µL from the initial stock solution were aseptically transferred to well A1 of the microtiter plate, that contained 100 µL of sterile MHB fractions, yielding a 50% dilution of the stock solution. Following comprehensive mixing of the contents in each well, 100 µL of the A1 fraction was transferred to the matching wells in B1, which additionally contained 100 µL of MHB fractions, followed by mix to achieve a further 50% dilution of the antibiotic. The previously indicated technique was repeated for each row to obtain additional dilutions.

2.5.1. Inoculum Preparation

Isolates were obtained from a bacterial culture on an overnight agar. An inoculum for MIC test was produced by selecting a minimum of three to five well-isolated colonies exhibiting identical morphology from the agar culture. A sterile loop was employed to contact the apex of each colony, and its culture was later transferred to a tube containing 4 to 5 ml of MHB. The culture was thereafter incubated at 35°C for approximately 2 hours until it reached a 0.5 McFarland standard. The turbidity of the actively proliferating broth culture was modified using sterile MHB or normal. The suspension was subsequently diluted 1:100 by mixing 100 µl of bacteria in suspension with (9900) µl of MHB, achieving a cell concentration of roughly 10^8 CFU/mL for the bacterial isolates.

2.5.2. Minimum inhibitory concentration (MIC) test

100 µL of a standardized suspension of bacteria at a concentration of 1 x 10^8 CFU/mL was introduced into each well containing 100 µL of pre-diluted antimicrobial drugs, yielding a final volume of 200 µL per well. A positive control was conducted in column 11 of the microplate to assess the sensitivity of the microbial isolates, which consisted of the broth and

bacterial inoculation. A broth without an inoculant was used as a negative control and was placed in column 12 of the microplate. The microtiter plates were then incubated overnight at 37°C for 24 to 48 hours. Following incubation, MIC (minimum inhibitory concentration) values were ascertained visually by dispensing 30 ml of Alamar Blue in the microplate wells and incubating at 37°C for 1 hour. Alamar blue was used as an indicator.

3 Results and discussion

3.1. X-Ray Diffraction (XRD) Studies

X-ray diffraction analysis has verified that the manganese dioxide nanoparticles possess a crystalline structure and are nanoscale in size. Figure 3 displays the XRD pattern of MnO_2 nanoparticles generated via a green technique utilizing Leek leaf extract. It demonstrates a wide pattern associated with bio-capped amorphous substances.

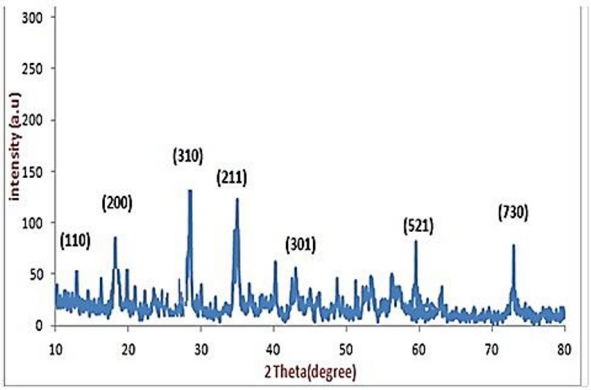

Fig.3. (XRD) pattern for manganese dioxide nanoparticles produced using *leek* leaf extract

Diffraction peaks have been detected at 2θ angles ranging from 10° to 80°, with a recognized orthorhombic structure in MnO2NPs corresponding to reflection values of 12.94°, 18.34°, 28.78°, 37.66°, 42.14°, 60.26°, and 73.72° (JCPDS no. 44-0141), and the strong XRD peaks indicate that MnO2 NPs have been successfully synthesized. According to Table (2), the mean size of MnO_2 nanoparticles was determined to be 39 nm.

Table 2. Crystalline sizes of MnO_2NPs derived from the extract of leek foliag

2θ (Deg.)	FWHM (Deg.)	Crystalline size D (nm)	hkl
12.94	0.2028	39.5	(110)
18.34	0.222	36	(200)
28.78	0.2976	27.6	(310)
37.66	0.2334	36.2	(211)
42.14	0.2	42.6	(301)
60.26	0.256	35.9	(521)
73.72	0.1800	55.2	(730)

3.2 Field Emission-Scanning Electron Microscopy (FE-SEM) Studies

Field emission scanning electron microscopy (FESEM) examines individual particles, including its aggregation, and may illustrate crystal structure, surface morphology, and distribution as well as aggregation of nanoparticles. Filed Emission-Scanning Electron Microscope (FE-SEM) analysis of the manganese dioxide (MnO2) nanoparticles prepared by Leek leaf extract revealed that the MnO2 NPs exhibit agglomeration polymorphic occurrence during the synthesis process. The MnO2 nanoparticles have a solid, compact morphology [9], as illustrated in Figure (4). The average grain size of nanoparticles ranged from 29 to more than 97 nm. The dimensions, morphology, and aggregation pattern of nanoparticles are contingent upon the quantitative presence of phytochemicals in plants. This might efficiently decrease, cap, and stabilize nanoparticles to specific sizes with irregular spherical forms, yielding heavily agglomerated nanoparticles [10].

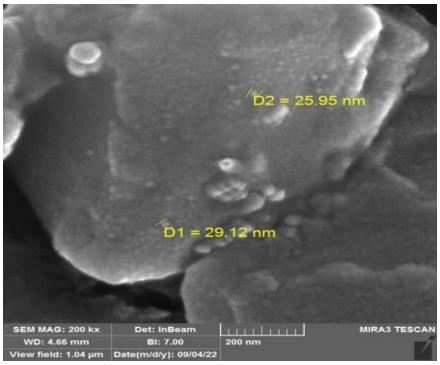

Fig.4. (FE-SEM) MnO2NPs using *Leek* leaf extract

3.3 Determination of Minimum Inhibitory Concentrations (MIC).

The antibacterial activity of resazurin was used as a confirmatory test (growth indicator) for the MIC test. In wells treated with 30 µg/ml or less of MnO2 nanoparticles, viable bacterial cells (pink color) were detected for both Staphylococcus aureus and S.mutans. Detection of non-viable cells (brown and blue color) for MnO2 nanoparticles synthesized from Leek leaf extract began at 1000-62.5 µg/ml of MnO2 nanoparticles (Figure 5). It was observed that the number of dead cells increased with increasing concentration. The results obtained in this test were similar to those for the MIC test using broth only. This result gives a good indication of the beneficial, more straightforward, and simpler use of resazurin in detecting the antibacterial activity of different nanomaterials. This result also gives information that the biosynthesis was determined to be more energy-efficient and capable of eliminating harmful compounds. Furthermore, the significant constituents in the plant extracts are hydroxyl and carbonyl groups, which function as reducing and stabilizing agents, explaining why the manganese oxide nanoparticles synthesized from the leek plant have good results.

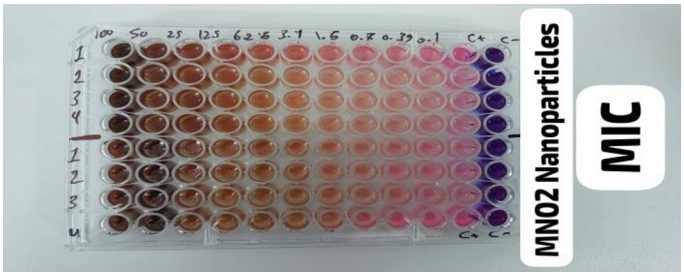

Fig. 5. The results of the broth microdilution technique for ascertaining minimum inhibitory concentration (MIC) values. (C-)" Negative control (only broth), (C+)" Positive control (only bacteria, broth), Wells with Blue color had no or inhibited growth, but Wells with red color with growth.

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