IHJPAS. 36 (3) 2023

Ibn Al-Haitham Journal for Pure and Applied Sciences

Journal homepage: jih.uobaghdad.edu.iq

Synthesis of Manganese Dioxide Nanoparticles by Plant Extract Mediated and their Effect on Biofilm Formation

Sabeeha K. Shanshool
Department of Physics, College of Sciences for Women, University of Baghdad, Baghdad, Iraq.

Zainab J. Shanan*
Department of Physics, College of Sciences for Women, University of Baghdad, Baghdad, Iraq.

***Corresponding author: zainabjs_phys@csw.uobaghdad.edu.iq**

Article history: Received 7 October 2022, Accepted 21 February 2023, Published in July 2023.

doi.org/10.30526/36.3.3061

Abstract

In the current work, Punica granatum L. peel, Artemisia herba-alba Asso., Matricaria chamomilla L., and Camellia sinensis extracts were used to prepare manganese dioxide (MnO_2) nanoparticles utilizing a green method. Energy-dispersive X-ray (EDX) analysis, Fourier Transform Infrared Spectroscopy (FTIR) analysis, and Filed emission-scanning electron microscopy (FE-SEM) analysis were used to evaluate the produced MnO_2 NPs. FE-SEM pictures demonstrated how agglomerated nanoparticles formed. According to FE-SEM calculations, the particle size ranged from 18.7-91.5 nm. FTIR spectra show that pure Mn-O is formed, while EDX results show that Mn and O are present. The ability to suppress biofilm growth in the produced MnO_2 NPs was examined. The outcomes showed that both bacterial and fungal biofilms were effectively inhibited by the MnO_2 NPs produced.

Keywords: Manganese dioxide NPs, biofilm inhibition, plant extracts.

1.Introduction

Serious worldwide concern has evolved in the form of antibiotic and antifungal treatment resistance in pathogenic bacterial and fungus species [1]. The production of biofilms by these microbes is a major factor in the antimicrobial medications' ineffectiveness [2]. These harmful microorganisms can tolerate 1,000 doses of traditional antibacterial drugs in the form of biofilms [3]. In addition, these harmful microorganisms have acquired resistance to antibiotics by creating efflux mechanisms, reducing cell wall penetration, changing the drug's target locations, etc [4]. Because these pathogenic bacteria species are responsible for various infectious disorders, these antimicrobial medicines are ineffective in treating them. By creating nanosized materials,

nanotechnology has arisen as a solution to the problem of antimicrobial resistance in this situation [5]. Medicine has been modernized with the introduction of nanotechnology, the most significant breakthrough in recent years. Nanotechnology, a groundbreaking science, will influence our efforts to improve human health. The medical industry has studied the longevity, efficiency, durability, flexibility, and inimitable physicochemical characteristics of nanoparticles. They are being utilized in numerous therapeutic approaches, such as the targeted delivery of medications, prognostic visual monitoring of therapy, and even tumor identification [6]. Nanoparticles (NPs) have become a useful weapon in the fight against bacterial biofilms. It is because NPs are immune to the processes that cause antibiotic resistance [7]. Metal oxide nanoparticles, including TiO2, Fe3O4, ZnO, MnO2, CuO, and several mixed metal oxides, are among the numerous NPs but are the most promising and extensively investigated [8]. Many metal oxide NPs have been shown to display biological characteristics far more favourably than the NPs of the parent metals. Because of this, the scientific community showed the most interest in metal oxide NPs [9]. NPs and other nanomaterials are gaining a lot of interest due to their distinctive physical properties. Furthermore, because of their physical properties, NPs are ideal for use in biological, electrical, sensing, and optoelectronic applications [10]. Gram-negative bacteria Escherichia coli, Klebsiella pneumonia, Gram-positive bacteria Staphylococcus aureus, Bacillus subtilis, and C. albicans, as well as biofilm development, were used to examine the antibacterial properties of manufactured Manganese dioxide nanoparticles.

2.Materials and methods:

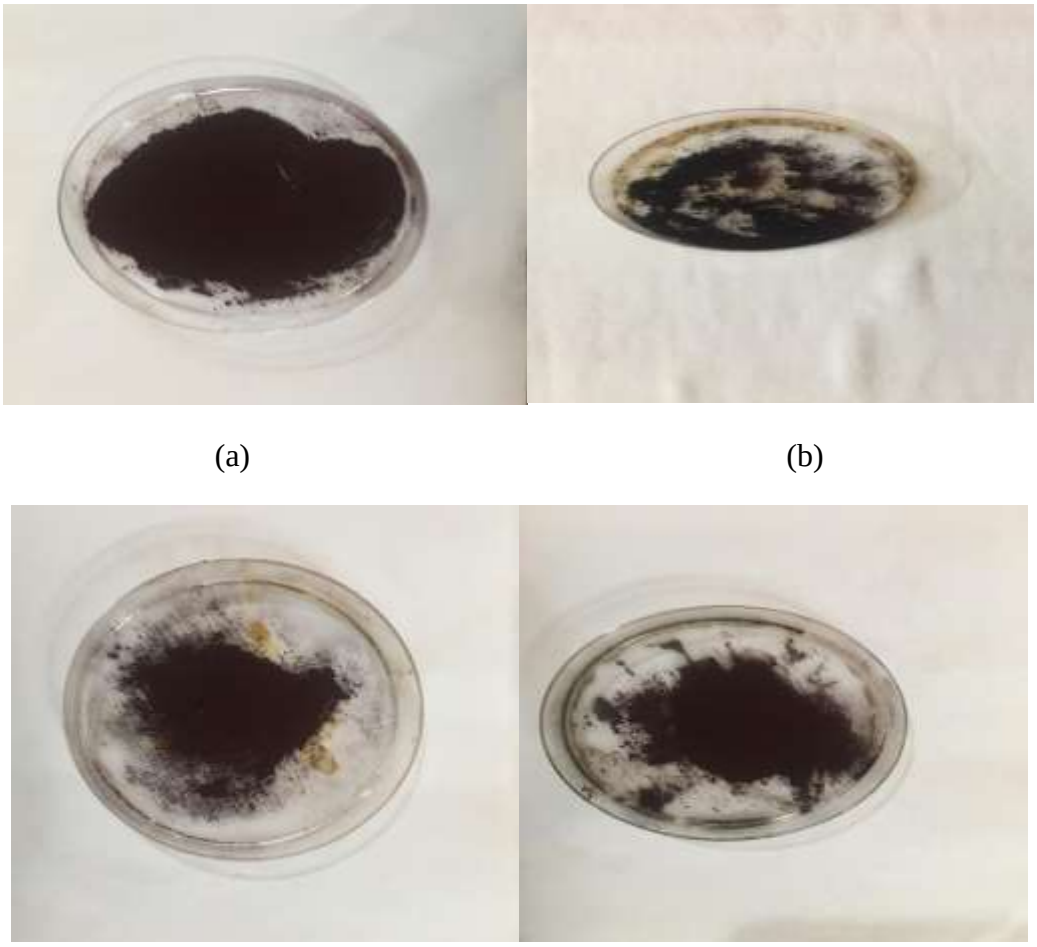
2.1. Green methods of synthesis Manganese dioxide nanoparticles

Preparation of hot aqueous extract

The leaf powder (10gm) of Punica granatum L. peel, Artemisia herba-alba Asso., Matricaria chamomilla L., and Camellia sinensis was mixed well with (150 ml) of distilled water boiled at 80°C and then homogenized on a magnetic stirrer for 2 hours, although the colour of the aqueous solution was different. The aqueous solution was then filtered and centrifuged at 6000 rpm for 20 min, then kept at 4°C until use.

2.2. The synthesis of MnO_2 NPs by using aqueous extract of Punica granatum L. peel ,Artemisia herba-alba Asso., Matricaria chamomilla L. and Camellia sinensis.

To make the precursor solution, combine 0.2g manganese acetate $(CH_3COOH)_2$ $MN.6H_2O$ with 100 ml of distilled water and heat for ten minutes with magnetic stirring. To make MnO_2 nanoparticles, 50 ml of the precursor solution was mixed with 5 ml of aqueous extracts from Punica granatum L. peel, Artemisia herba-alba Asso., Matricaria chamomilla L., and Camellia sinensis extracts on a magnetic stirrer at 70°C for 60 minutes, despite the fact that the colour of the aqueous solution varies. The hue of the solution changed to brown when manganese acetate was added to both Punica granatum L. peel, Matricaria chamomilla L. and Camellia sinensis extracts. The hue of the solution changed to dark brown when it was mixed with extracts of Artemisia herba- alba Asso. After that, the sample was centrifuged for 15 minutes at 8000 rpm. The solution was baked for 2 hours at 250°C, also carefully collected and kept the powder for characterization purposes. (See **Figure1**).



(a) (b)

(c) (d)

Figure1. Manganese dioxide nanoparticles green synthesized via (a) Punica granatum L. peel (b)Artemisia herba-alba Asso. extract (c) Matricaria chamomilla L. extract (d) Camellia sinensis extract.

2.3. Biofilm formation method

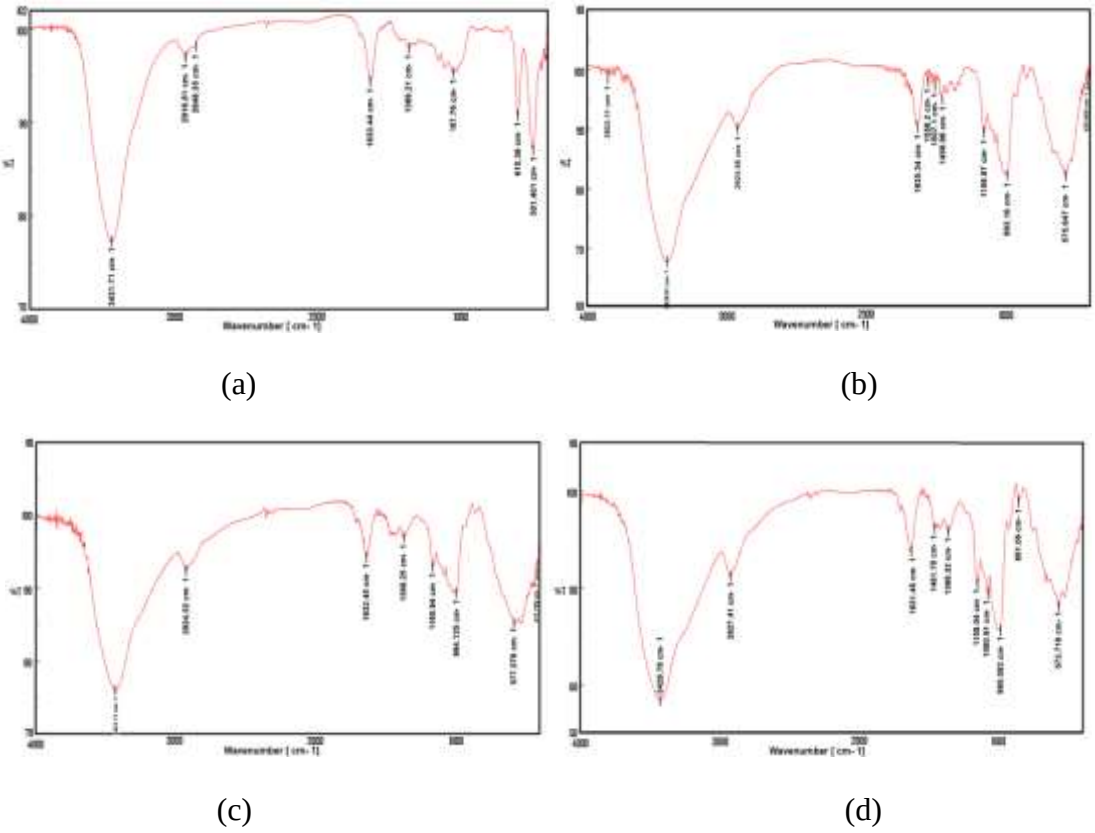
Biofilm formation assays were performed using a 96- well microtiter plate, based on the protocol by Goh, S. et al. (2013) [11], different bacterial strains were cultured in TS broth for an overnight period before being diluted to 1:100 (TSB + 1% w/v glucose). Except for the control well, which was left empty, the microtiter plate's wells were each filled with 100 ml of medium and 100 MnO2 NPs. Following that, the plate was incubated for 24 hours at 37 °C, poured into the wells, stained for 10 minutes at room temperature, and then washed off by immersing the plate in a water tray and letting it dry in the air. The optical density (OD) of the wells was measured in a tiny plate reader at 630 nm after the wells were stained for 10 minutes at room temperature with 95 percent ethanol [12].

3. Characterization

The synthesized MnO_2 nanoparticles are subjected to various characterization studies to study their structural and optical properties, such as Fourier Transform Infrared Spectroscopy (FTIR), Filed Emission- scanning electron micrographs (FE-SEM), and energy dispersive X-ray (EDX). The results obtained are discussed below.

3.1.FTIR Analysis

The prepared manganese oxide nanoparticles were also subjected to FT-IR spectroscopy. Stabilized manganese nanoparticles' FTIR spectrum is seen in (Fig. 2. The spectra were captured between 1000 and 4000 cm1. The FTIR spectrum has recognizable peaks. According to the findings, the peak between (3428.81 and 3431.71) cm^{-1} can be attributed to the O-H stretching of any ethanol or water that is present in the system. Due to interaction with manganese dioxide nanoparticles, secondary metabolites in plant extracts exhibit C=O stretching between (1631.48 and 1635.34) cm^{-1}. The absorption peak between (1360.21-1368.25) cm^{-1} demonstrated the bending band of adsorbed water of Mn nanoparticles. Peaks located between (1057.76 and 1160.04) cm^{-1} were used to identify the (C-O) band presence that is present in plant extracts. The MnO_2 nanoparticles were confirmed to be present in the sample by the substantial absorption peaks between 993.16 and 995.089 cm^{-1} that matched the distinctive stretching bonds O-Mn-O. As a result of our findings, green-synthesized MnO_2 NPs are completely capped with biologically active phytomolecules from plant extracts with such functional groups [13].



(a) (b)

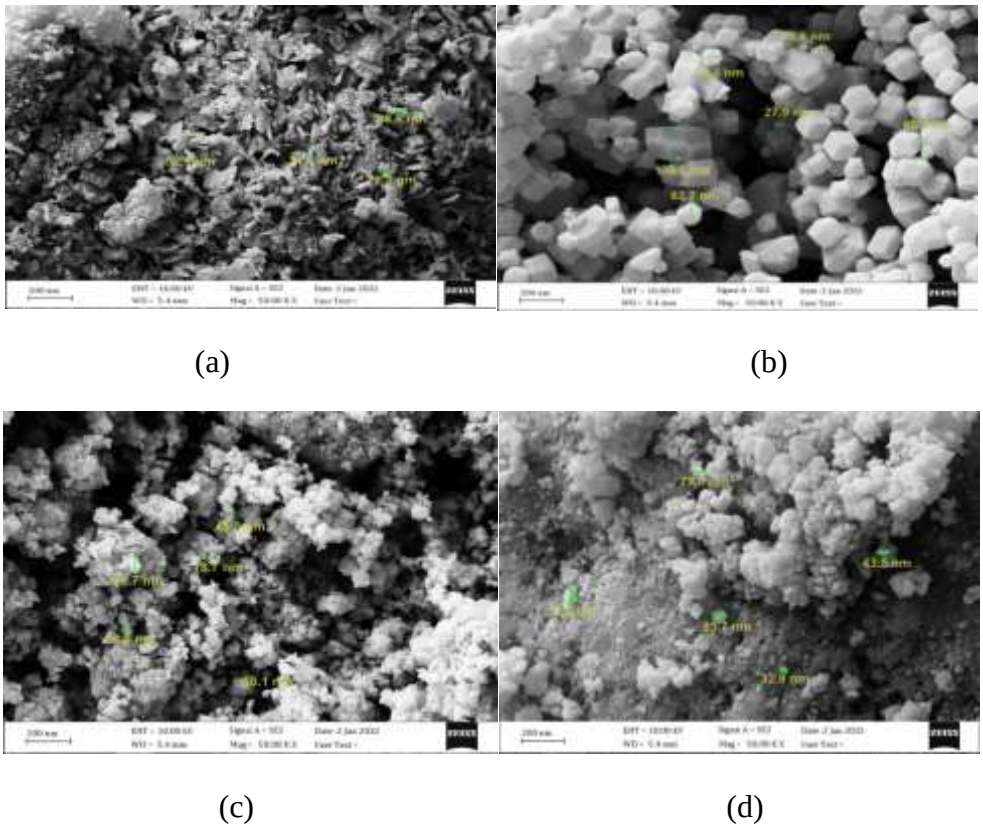
(c) (d)

Figure 2. FTIR Analysis of green-synthesized of MnO_2 NPs using (a) Punica granatum L. peel extract (b)Artemisia herba-alba Asso. extract (c) Matricaria chamomilla L. extract (d) Camellia sinensis extract.

3.2. FE-SEM Analysis

Manganese dioxide nanoparticles made from Punica granatum L. peel extract were examined using a field Emission-Scanning Electron Microscope (FE-SEM), and the results showed that during the synthesis process, polymorphic agglomeration occurred. The particle size was found to be in the range of (34.1-89.6) nm (**Figure 3a**) according to the surface morphology of the MnO_2 NPs by Artemisia herba-alba Asso. As shown in Fig. 3b, the particles were in cubic form and in different sizes, The particle size was in the range of (20.6–82.7) nm. In (**Figure 3c**) shows the FE-SEM of

MnO_2 NPs using Matricaria chamomilla L. extract, the particle size is found in the range (18.7-79.3) nm, which are small and large lumps combined with each other and with irregular shapes. synthesized in green from MnO_2 NPs via Camellia sinensis extract, they are lumps of various sizes, have a spongy texture, and The particle size ranged from (32.9-91.5) nm, as shown in (Fig.3d).



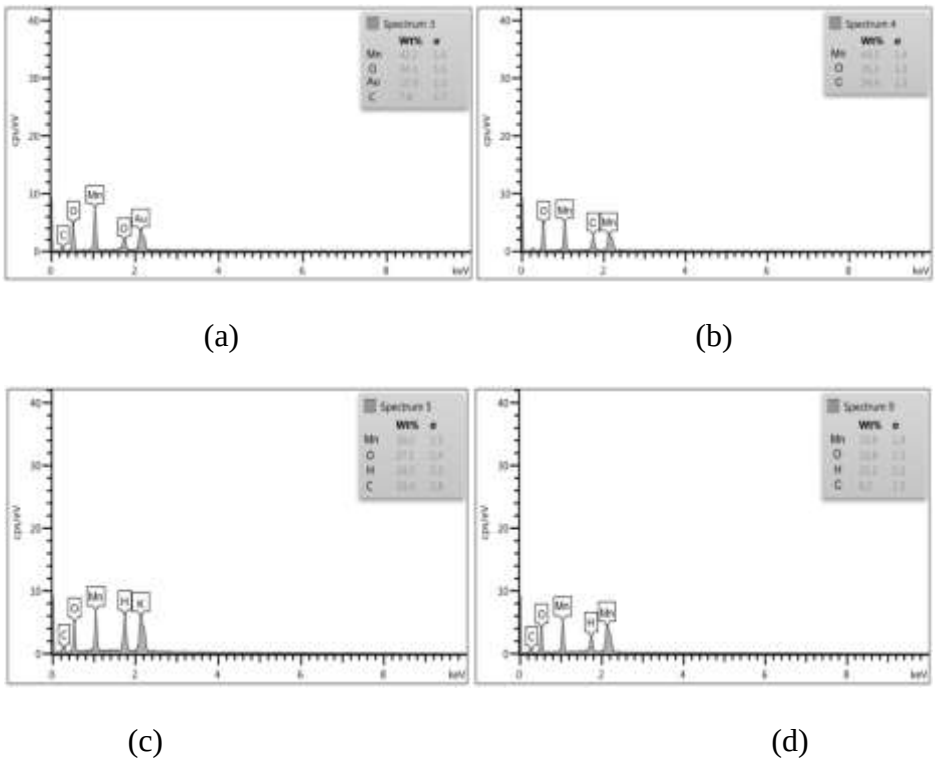
(a) (b)

(c) (d)

Figure3. FE-SEM Analysis of green-synthesized of MnO_2 NPs using (a) Punica granatum L. peel extract (b)Artemisia herba-alba Asso. extract (c) Matricaria chamomilla L. extract (d) Camellia sinensis extract.

3.3. EDX analysis

The chemical structure of the green composite MnO_2 NPs were investigated using EDX analysis. The successful green synthesis of NPs was validated using biomolecules from Punica granatum L. peel, Artemisia herba-alba Asso., Matricaria chamomilla L., and Camellia sinensis by EDX pattern and EDX mapping (**Figure 4**). The EDX peaks at 1.2 keV and 2.4 keV in the EDX spectrum confirmed the presence of the chemical element (Mn) in the produced NPs [14]. The presence of the carbon (C) and oxygen (O) peaks in the EDX spectrum, in addition to the Mn EDX peaks, confirmed the adsorption of biological molecules on the surface of NPs made from plant extracts. The EDX results further confirmed that the MnO_2 NPs contained very few impurities. As a result, the EDX results show that plant extracts have successfully been used to synthesize NPs of interest. The EDX patterns of MnO_2 NPs synthesized in green are consistent with those previously reported in the literature [15].



(a) (b)

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Figure4. EDX analysis of green-synthesized of MnO_2 NPs using (a) Punica granatum L. peel extract (b)Artemisia herba-alba Asso. extract (c) Matricaria chamomilla L. extract (d) Camellia sinensis extract.

3.4. Biofilm Inhibition Investigations

MnO_2 NPs were tested for their biofilm inhibitory effect against infectious bacterial and fungal species using leaf extracts from Punica granatum L. peel, Artemisia herba-alba Asso., Matricaria chamomilla L., and Camellia sinensis. According to the findings, MnO_2 NPs have significant net biofilm inhibitory activity, inhibiting the formation of biofilms in both bacterial and fungal strains. Moreover, as shown in Fig. 5, the green production of MnO_2 using Punica granatum L. peel and Artemisia herba-alba Asso. extracts showed the highest percentage of biofilm suppression activity, while the activity of MnO_2 NPs prepared by Camellia sinensis extract was the lowest among the rest of the extracts, as shown in Table 1. In addition, we tested the biofilm inhibition activity data against both fungal strains employing green production of MnO_2 using Punica granatum L. peel, Artemisia herba-alba Asso., Matricaria chamomilla L., and Camellia sinensis extracts, which demonstrated a substantial difference, where the highest percentage of MnO_2 NPs was prepared by Artemisia herba-alba Asso. extract, as shown in Table 2. The expression of cell wall proteins can change the surface of the cell wall from hydrophilic to extremely hydrophobic toward nanoparticles, which can affect the tolerance or susceptibility of bacteria and fungi to nanoparticles and diffusion within biofilm matrixes [16].

Table 1. the effect of MnO_2 NPs on bacterial biofilm formation.

Bacterial isolates	**control**	**Treatment by samples**			
		9	10	11	12
Bacillus Subtilus	0.256	0.095	0.143	0.175	0.188
Staphylococcus aureus	0.255	0.110	0.089	0.115	0.139
Escherichia coli	0.471	0.288	0.314	0.357	0.398
Klebsiella pneumonia	0.505	0.389	0.393	0.415	0.477

Where the numbers (9, 10, 11 and 12) refer to manganese dioxide nanoparticles prepared by the extracts (Punica granatum L. peel, Artemisia herba-alba Asso., Matricaria chamomilla L., and Camellia sinensis) respectively.

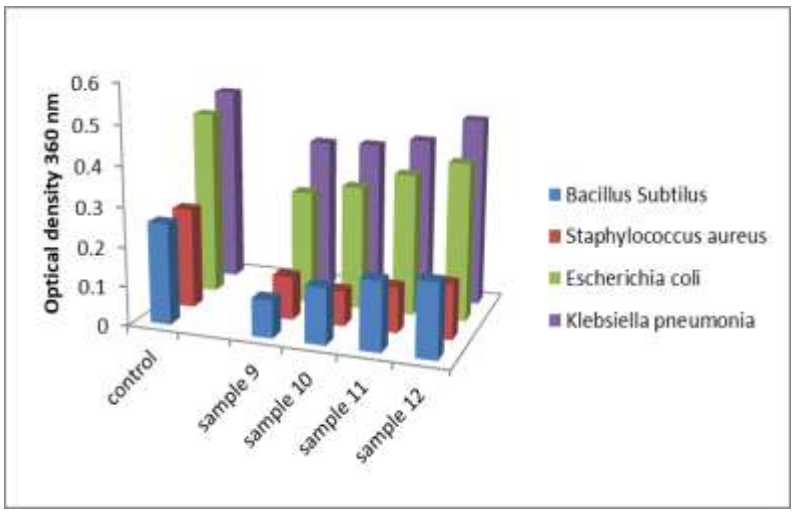

Figure5. After treatment with different MnO_2 NPs bacteria's biofilm development differed.

Table 2. the effect of MnO_2 NPs on biofilm growth on fungi

		Treatment by samples			
Fungal isolates	**control**	9	10	11	12
C.albicans	0.684	0.453	0.434	0.456	0.516

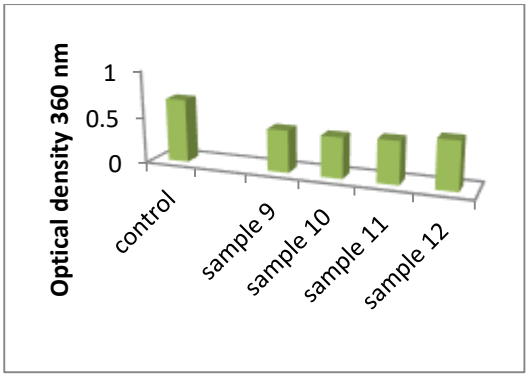

Figure6. C. albicans biofilm development discrepancies following treatment with various MnO_2 NPs.

4.Conclusion

The plant extracts of Punica granatum L. peel, Artemisia herba-alba Asso., Matricaria chamomilla L., and Camellia sinensis demonstrated the ability to produce manganese dioxide nanoparticles. With the use of several spectroscopic methods, the produced MnO_2 NPs were effectively characterized. The creation of pure and crystalline MnO_2 nanoparticles with high purity and crystallinity, decreased particle size, and increased surface flaws is confirmed by FE-SEM and FTIR. These characteristics make these particles favorable for usage in a variety of environmental and application settings. EDX spectrum shows the Mn & O nanoparticles are present in the prepared nanoparticles. The ability to prevent biofilms with the produced MnO_2 NPs was examined. The outcomes showed that both bacterial and fungal biofilms were effectively inhibited by the produced MnO_2 NPs.

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