

## Green Synthesis, Characterization of Manganese Oxide Nanoparticles using Ziziphus Abyssinia Plant Extract and their Antimicrobial Efficacy

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### Abstract

Development of green technology is generating interest of researchers towards eco-friendly and low-cost methods for biosynthesis of nanoparticles (NPs). In this study, manganese oxide (MnO) NPs were synthesized using a manganese acetate precursor and Ziziphus Abyssinia leaves extract. The biosynthesized MnO NPs were characterized using an X-ray diffractometer (XRD), scanning electron microscopy (SEM), Ultraviolet visible spectroscopy (UV-Vis), and Fourier transform infrared (FTIR) spectroscopy. XRD characterization confirmed that the biosynthesized MnO NPs possessed a good crystalline nature which perfectly matched can be assigned to tetragonal structure. FTIR spectra of MnO NPs revealed the presence of O-Mn-O stretch vibration at around 913.80 and 796 cm<sup>-1</sup>. Furthermore, the results obtained from SEM showed that the biosynthesized MnO NPs were spherical in shape which is also in agreement with that reported by Jayandran et al. (2015). Moreover, the antimicrobial activities of different concentrations of MnO NPs synthesized using Ziziphus Abyssinia extract were also tested. From the inhibition zone results, synthesized MnO NPs were showed better inhibition activity than the Ziziphus Abyssinia leaf extract against Escherichia coli, Staphylococcus aureus Salmonella Typhi, Shigella bacteria and also exhibited similar inhibition activity to standard drug against Candida albicans, Candida Tropicalis, Aspergillus Niger and Aspergillus flavus. Thus, our findings report Mn nanoparticles synthesized from the above proposed green method are show promise results in the view of pharmaceutical and therapeutic applications.

**Keywords:** Fungal strain, green chemistry, bacteria, Biosynthesis, Nanotechnology, Ziziphus Abyssinia.

### Introduction

In the last ten years, scientists from all around the world have become increasingly interested in using nanomaterials to regulate microbial proliferation. Increased health issues are a result of microbes becoming more resistant to antimicrobial drugs, including antibiotics. Numerous studies have shown that novel uses for metal nanoparticles can be found by fusing the three forces of material science, nanotechnology, and the built-in antibacterial properties of some metals. Numerous studies have documented the toxicity of metal and metal oxide nanoparticles toward a variety of bacteria. It is possible to effectively use these nanoparticles to halt the growth of different bacterial species [1]. Numerous studies have been carried out in an effort to improve the current antimicrobial therapies as a result of the spike in the creation of multi-drug resistant microorganisms, which is presenting a serious challenge for public health. It has been determined that one or more of the first- and second-line medications that have historically been used to treat the infection have developed resistance in about 70% of bacterial infections. The creation of innovative

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antimicrobial drugs that are also effective must be developed and synthesized faster than ever before. Nanoparticles as antibacterial agents have shown to be an innovative solution to this problem since they have the capacity to create a strong nanostructure that can be used to deliver the antibacterial agents, effectively and locally targeting bacterial growth. Additionally, nanoparticles have shown to be so powerful that viruses have little opportunity to develop a resistance to them. At the doses that have been used to kill bacterial cells, the majority of the metal oxide nanoparticles that are now available are not harmful to mammalian cells, which makes employing them on a wider scale advantageous. It has been established that metal nanoparticles with antibacterial activity include Gold (Au), Silver (Ag), Silicon (Si), Silver Oxide (Ag<sub>2</sub>O), Titanium Dioxide (TiO<sub>2</sub>), Zinc Oxide (ZnO), Copper Oxide (CuO), Calcium Oxide (CaO), And Magnesium Oxide (MgO).

The majority of current methods for creating metal nanoparticles often rely on physical or chemical principles. However, neither preparation technique is eco-friendly. The integration of nanotechnology with green chemistry will be popular in the coming decades. Nanotechnology is a ground breaking science that is still in its early stages. Green synthesis has emerged as an alternative to the limitations of traditional technologies. Green synthesis focuses primarily on the disposal of hazardous wastes, the use of environmentally friendly chemicals, solvents, and renewable resources, as well as sustainable procedures [2]. The manufacture of various metal nanoparticles utilizing green nanotechnology has been reported to use yeast, fungi, bacteria, algae, plant extract, etc.

Because using plants to synthesize nanoparticles is proving to be more advantageous than using microbes due to the presence of a wide variety of bio-molecules in plants that can act as capping and reducing agents, plant extracts used in reduction methods can be considered to be more effective green approaches for synthesizing metal nanoparticles [3,4]. Thus, under benign experimental conditions, such as relatively low reaction temperature and ambient pressure, improves the rate of reduction and stability of nanoparticles and synthesis can be carried out. Due to their superior physicochemical qualities, manganese oxides can be used in a variety of applications, including batteries, magnetic materials, water treatment, and imaging contrast agents. One of the most significant materials is MnO<sub>2</sub>, and many researchers are interested in how adding MnO<sub>2</sub> affects the electromagnetic properties of ferrite materials. MnO<sub>2</sub> created at the nanoscale has been prepared using a variety of techniques. Although there are numerous reports of environmentally friendly manganese nanoparticle production, the simplest, most affordable, and environment-friendly technique is to reduce and stabilize Mn metal into nanoparticles using plant extracts, as was stated above. The well-known medicinal ingredient from the *Ziziphus Abyssinia* plant has been found to offer a variety of therapeutic effects. Additionally, it has been claimed that the fruits of this plant contain antifungal, antibacterial, antiulcer, and anti-inflammatory properties [5,6].

Most of the reports are focusing on the characterization and application of the formed manganese nanoparticles in catalytic activity, electronic properties, but the antimicrobial effects of manganese nanoparticles are investigated rarely. Based on the foregoing discussions, this inquiry is concerned with the environmentally friendly synthesis of Mn nanoparticles utilizing *Ziziphus Abyssinia* leaf extract. The biological applications of Mn nanoparticles, such as antibacterial and antifungal properties against some bacterial and fungal strains, are the main emphasis of this work.

## **Materials and Methods**

### **Sample Preparation**

*Ziziphus Abyssinia* leaves were collected from Yola South of Adamawa State. The sample was washed thoroughly with distilled water to remove dust allowed to dry at room temperature on a clean polythene plastic bag for at least two weeks. The dried leaves were grounded mechanically using sterile pestle and mortar. The sample was sieved, packed in polyethylene bags labelled and

stored for phytochemical screening, extract preparation for synthesis of Manganese oxide nanoparticles and Soxhlet extraction for anti-microbial activity analysis.

### **Plant extract preparation**

10 g of plant materials was boiled in 250 ml distilled water for 2 min. The plant materials were filtered using Whatman no. 1 filter paper and the extracts were centrifuged at 3500 rpm for 15 min and the filtrated extract was dried by evaporating the water with a rotary evaporator. 150 mg of the extract was dissolved in 10 ml distilled water and was stored in an amber bottle, at 10°C until utilisation for the green synthesis of manganese. For leaf extraction, 20 g of *Ziziphus* plant was subjected to the Soxhlet apparatus and were extracted with 300 ml of 95% ethanol for 5 h. The extract was dried at 60°C. 50 mg of the extract was dissolved in 10 ml ethanol and stored in an amber bottle, at 10°C until further uses.

### **Green synthesis of MnO Nanoparticles**

Aqueous solutions of manganese acetate (0.01 M) were prepared in different pH values (4 - 8). Different volumetric ratio of manganese solution and extracts (15 mg/ml) were mixed (extracts/metal: 10:90, 25:75, and 50:50 v/v). The mixtures remained for 40, 80 and 120 min, and then the fresh extract of *Ziziphus* (5 mg/ml) was added to the solutions. The samples were centrifuged at 3500 rpm for 15 min. The NPs were separated from the solutions and then washed for several times using ethanol and distilled water. The supernatant was decanted and kept in oven to dryness.

### **Characterization of MnO Nanoparticles**

#### **UV-vis spectral analysis**

The UV-vis spectroscopy is commonly used to characterize different metal NPs in the size range of 2 nm–100 nm . Synthesis of the NP was confirmed by scanning an aqueous solution by UV- vis spectrophotometer at 200 nm–800 nm. All UV-vis spectroscopic measurements of the synthesized MnO nanoparticles were carried out on Perkin Emier, Lambda 25 UV/vis spectrometer.

#### **Fourier Transform Infrared (FT-IR) spectrum analysis**

Infrared spectra were recorded by FTIR spectroscopy. 1 mg of the synthesized nanoparticles was mixed with 200 mg KBr and was pressed into a pellet. Infrared spectra were recorded using a (Perkin Elmer – Spectrum 65) FT-IR spectroscopy, from 4000 to 400 cm<sup>-1</sup>.

#### **Scanning Electron Microscopy (SEM)**

Scanning Electron Microscopy (SEM) with a secondary electron detector can visualize crystal shape, surface morphology, dispersed and agglomerated nanoparticles, and surface functionalization's SEM can examine each particle, including the aggregation of particles particle.

### **Preparation of the broth and microorganism suspensions for antimicrobial activity**

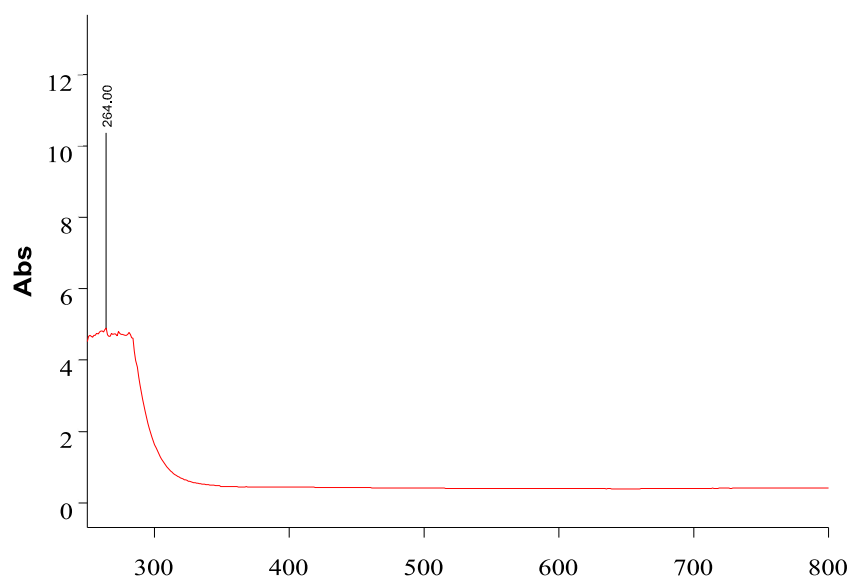
The antibacterial activity of extract of *Ziziphus Abyssinia* leaf extract and nanoparticles was determined by agar well diffusion method. Microorganism suspensions were prepared according to the 0.5 concentration with *Escherichia coli*, *Staphylococcus aureus* *Salmonella Typhi*, *Shigella* bacteria and *Candida albicans*, *Candida Tropicalis*, *Aspergillus Niger* and *Aspergillus flavus* [11]. A Muller Hinton liquid medium was used for the bacteria, while an RPMI medium was used for fungi. Media, nanoparticle and microorganism suspensions were added to the microplates and the inoculated cultures were incubated at 30°C for 24 h. The

antimicrobial effects of the commercially available Augmentin and Griseofulvin antibiotics were also examined for comparison.

## Results and Discussion

### UV-Visible spectroscopy

The UV–vis absorption intensity of nanoparticles depends on its concentration which generally increases with the increase of its concentration and an increase in the absorption intensity indicates better solubility and dispersion of the NPs in. UV–vis spectroscopy of MnO nanoparticles **FIG 1**, shows the maximum absorption at 264 nm. This is because of  $n \rightarrow \pi^*$  transition or  $n \rightarrow \pi^*$  and  $\pi \rightarrow \pi^*$  transitions. Different place of the absorption bands shows that different morphologies and size variations are presented. The results marks that the most effective parameter was the ratio of the extracts to the metal and illustrates that the higher ratio of the extract to the metal causes the synthesis of NPs to increase while increasing the time and decreasing the pH provides a minor positive effect. The intensity of the band is seen to be a function of the amount of plant extract used in the reaction and the increase in the extract concentration led to an increase in the synthesis of MnO nanoparticles. According to most of the MnO nanoparticles were synthesized in 30 min and further time did not affect the reaction [7,8].



**FIG 1. UV-Visible spectra of MnO nanoparticles.**

### Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR spectroscopy was used to identify the responsible functional group existing in the biomolecules of the plant extract to reduce the manganese ions. A spectrum of the MnO nanoparticles is shown in **FIG 2**. The broad peak at  $3447 \text{ cm}^{-1}$  corresponds to an O–H band stretching vibration presence in the system. And also, typically the peaks at  $913.80 \text{ cm}^{-1}$  and  $796 \text{ cm}^{-1}$  may correspond to O–Mn–O bond which demonstrated the presence of the  $\text{MnO}_2$  nanoparticles in the sample (Souri et al., 2018). The peaks at  $3018 \text{ cm}^{-1}$  -  $2863 \text{ cm}^{-1}$  relates to  $-\text{C}=\text{C}$  bond,  $1464 \text{ cm}^{-1}$  -  $1580 \text{ cm}^{-1}$  peaks relate to the aromatic  $\text{C}=\text{C}$  bond of the plant extract which surrounds the NPs and prevents them from agglomeration. The  $\text{C}=\text{O}$  bond of the plant appears at  $1650 \text{ cm}^{-1}$ . The  $\text{C}-\text{O}$  band of plant extract was assigned by the peak at  $1255 \text{ cm}^{-1}$ . FT-IR spectra of the extract also show the OH bending modes of the phenolic compounds of the extract which are responsible for the reduction of ions. Comparison of the spectra of the extracts and MnO NPs indicates the effect of the extracts in the synthesis of the nanoparticles. This result is similar to the report of on the

synthesis of MnO nanoparticles using *D. graveolens* extract [9].

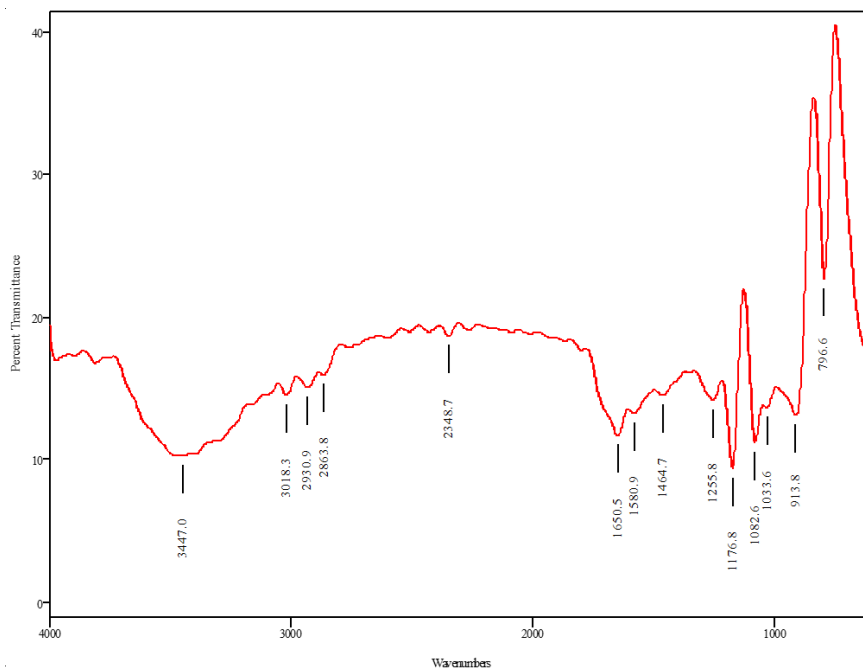


FIG .2. FT-IR spectrum of MnO nanoparticles

### X-ray Diffraction (XRD) Studies

Figure 3 show the XRD pattern of prepared manganese oxide nanoparticles. The sharp peak of XRD exhibits the prepared samples were highly crystalline in nature. All observed peaks can be indexed to a pure tetragonal structure. The diffraction peaks of manganese oxide nanoparticles prepared from various manganese salts of two different anions, exhibits at  $2\theta^\circ$  angles of 21.4957, 23.8331, 40.3036, 42.1860, 42.9910 and 44.1446 **FIG. 3** that correspond to the (110), (111), (101), (210), (220) and (002) that can be assigned to tetragonal structure. The obtained results are in well agreement with the JCPDS pdf number of 10799. It concludes that there is no difference in structural properties when the precursor is changed [10].

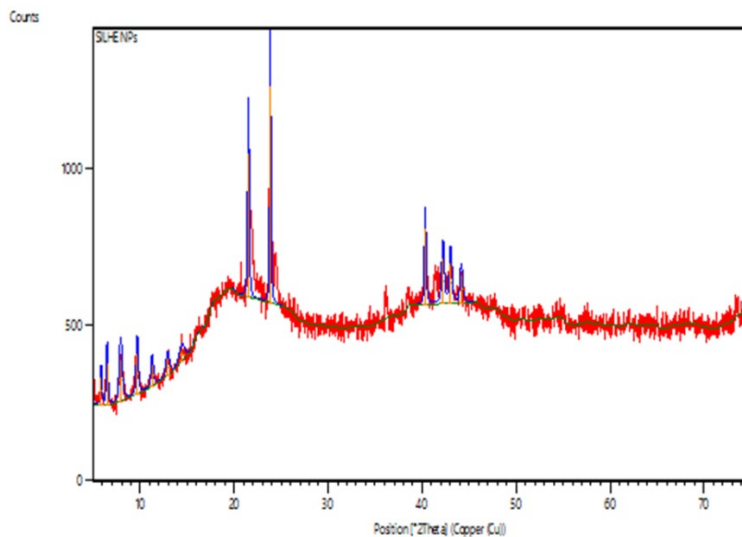


FIG 3. XRD pattern of MnO nanoparticles

### Scanning Electron Microscopy

Morphology of synthesized manganese oxide nanoparticles was characterized by SEM analysis. The SEM images of manganese nanoparticles are shown in **FIG.4**, which exhibits the agglomeration occurred during the synthesis process. It can be view that the MnO NPs formed are moderately dispersed and slightly agglomerated. SEM images of those compounds had shown very clear that most of the particles are polymorphic morphology of material.

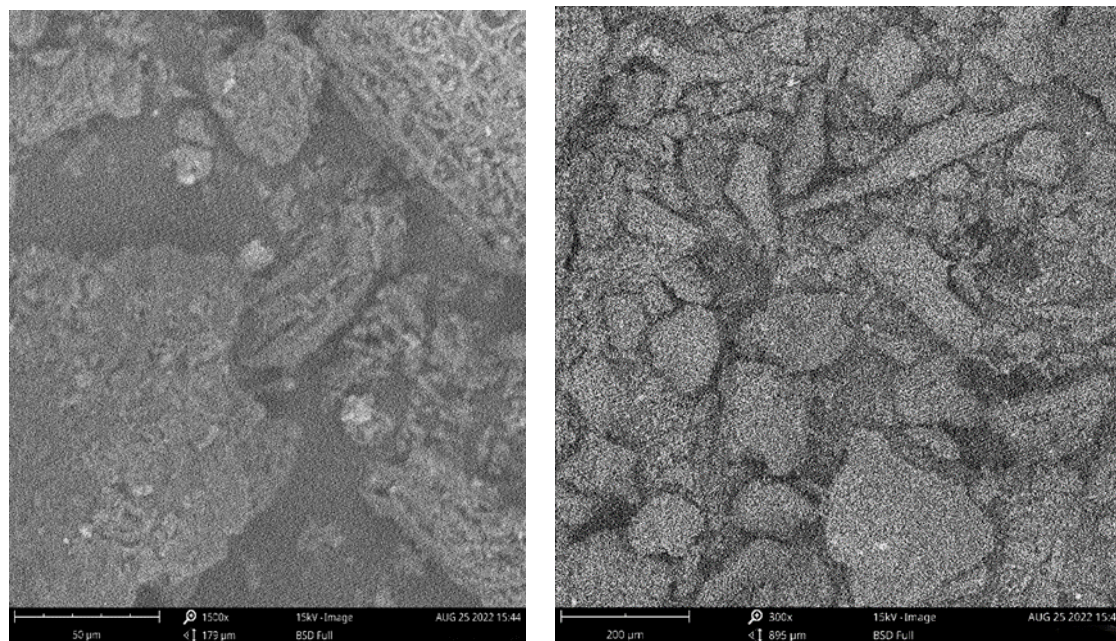


FIG 4. SEM monographs of MnO nanoparticles.

### Antimicrobial Activity

#### Antibacterial Activities of Extract and Manganese Oxide Nanoparticles.

By using the well diffusion method, the antibacterial efficacy of an aqueous leaf extract against *E. coli*, *Staphylococcus aureus*, *Salmonella typhi*, and *Shigella* was assessed. Spread plate inoculation was used to inoculate the cultures [11]. The same process was used to determine the manganese oxide nanoparticles from the *Ziziphus Abyssinia* plant's antibacterial activity. The plates were then kept at 30° C for the following 24 hours. According to results in **TABLE 1**, nanoparticles are more effective than leaf aqueous extracts. While leaf extract shown less inhibitory zones against several bacteria, the nanoparticle demonstrated more. The nanoparticles had a maximum zone of inhibition of 26 mm against *E. coli*, whereas the leaf extract had a maximum zone of inhibition of 10 mm. When we used *Staphylococcus aureus* bacteria, the nanoparticles showed a maximum zone of inhibition of 23 mm, whereas leaf extract showed a zone of inhibition of 13 mm; similarly, the nanoparticles showed a maximum zone of inhibition of 19 mm when we used *S. typhi* bacteria, whereas leaf extract showed a zone of inhibition of 12 mm. While leaf extract demonstrated a 15 mm zone of inhibition against *Shigella* bacteria, nanoparticles demonstrated a 19 mm zone of inhibition.

TABLE 1: The antibacterial activity of the tested aqueous extract of *Ziziphus* and Manganese Oxide nanoparticles

Bacteria	Aqueous extract			MnO NPs		
	500ug/ml	250ug/ml	125ug/ml	500ug/ml	250ug/ml	125ug/ml
<b>E. Coli</b>	10 mm	6 mm	6 mm	21 mm	10 mm	26 mm
<b>Staph. Aureus</b>	13 mm	11.5 mm	11 mm	10 mm	6 mm	23 mm
<b>Salmonella typhi</b>	12 mm	6 mm	6 mm	17 mm	12 mm	19 mm
<b>Shigella</b>	15 mm	13.5 mm	8 mm	18 mm	15 mm	19 mm

**Determination of Antifungal Activities of Extract and Manganese Oxide Nanoparticles**

Using the agar well diffusion assay method, the synthetic MnO nanoparticles' antifungal activity was assessed. The information in TABLE 2, demonstrates that there are greater zones of inhibition against fungi in the nanoparticle of the *Ziziphus Abyssinia* plant when compared to leaf extract against several types of fungus. The maximal zone of inhibition for *Candida albicans* fungus by leaf extract nanoparticles was 11 mm, whereas the maximum zone of inhibition for MnO nanoparticles was 21 mm. While MnO nanoparticles demonstrated a 12 mm zone of inhibition, leaf extract only showed a maximum 8 mm zone of inhibition against *Candida tropicalis* fungus. Similar to this, the nanoparticles demonstrated a maximum 26 mm zone of inhibition against the same fungi while the leaf extract only showed a maximum 6 mm zone of inhibition. According to the table, the maximum zone of inhibition for leaf extract nanoparticles against the fungus *A. flavus* was 9 mm, whereas the maximum zone of inhibition for MnO nanoparticles was 23 mm.

TABLE 2: The antifungal activity of the tested aqueous extract of *Ziziphus Abyssinia* and Manganese Oxide nanoparticles.

Bacteria	Aqueous extract			MnO NPs		
	500ug/ml	250ug/ml	125ug/ml	500ug/ml	250ug/ml	125ug/ml
<b>Candida albicans</b>	11mm	9mm	8mm	14 mm	11 mm	21 mm
<b>Candida Tropicalis</b>	8mm	8mm	7mm	11 mm	9 mm	28 mm
<b>Aspergillus Niger</b>	6mm	6mm	6mm	13mm	8mm	26 mm
<b>Aspergillus</b>	9mm	6mm	6mm	10mm	8mm	23 mm

<b>flavus</b>						
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## CONCLUSION

Ziziphus Abyssinia leaf extract was employed in this experiment to make MnO nanoparticles. This strategy is effective, rapid, and affordable. We deduced from the XRD that the produced manganese oxide nanoparticles had a tetragonal structure and were extremely crystalline in nature. The SEM examination revealed the morphology of synthesized MnO nanoparticles to be spherical in shape. FTIR spectra show the effect of plant extracts on the extraction of NPs. The antibacterial and antifungal properties of MnO nanoparticles were better than that of leaf extract of *Ziziphus Abyssinia*.

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