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RESEARCH PAPER

Development of a Sustainable Bacterial Inhibitor Through Green Precipitation Utilizing Beetroot Extract of ZnO and MnO NPs

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ABSTRACT

This study deals with the preparation of new metal oxide materials derived from metal precursors using a low-cost sustainable green synthesis approach based on natural plant extracts. The preparation process involved the formation of powders of metal oxides by the precipitation method by adding Beetroot (Beta vulgaris) to Ionic aqueous solutions containing zinc chloride and manganese chloride. Different weight ratios of mineral precursors have been used to study the effect of chemical composition on the synthetic and functional properties of the resulting materials. After that, the prepared mixtures were subjected to heat treatment inside an electric oven to obtain powders of (ZnO) and manganese oxide (MnO). The final weight composition of the prepared materials was approximately 38% by weight of ZnO and 62% by weight of MnO, depending on the stoichiometric conversion of the initial salts to their corresponding oxides. The compositional, morphological and compositional characteristics of the prepared sample were studied using a number of advanced analysis techniques, including Fourier Transform Infrared Spectroscopy (FT-IR), field emission scanning electron microscopy (FESEM), and energy X-ray scattering spectroscopy (EDS). The FT-IR results confirmed the formation of the characteristic metal-oxygen bonds indicative of the formation of ZnO and MnO, while the FESEM images showed a morphological structure at the nanoscale with a heterogeneous distribution of particles influenced by the mechanism of green synthesis adopted in the preparation. The EDS analysis also confirmed the presence of the basic elements of zinc, manganese and oxygen inside the formed structure with a noticeable absence of impurities

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INTRODUCTION

Bacteria that are resistant to more than one drug (MDR) are now one of the biggest problems in medicine and public health around the world. Standard chemical disinfectants and antibiotics do work, but they can also harm the environment and make microbes stronger. Because of this, we need to quickly make "eco-friendly" antimicrobial inhibitors from safe, long-lasting, and biological sources like phytochemicals and green-synthesized nanomaterials [1]. Coordination chemistry and materials science might be able to help us reach

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this goal by making metal-based complexes [2]. Two transition metals that are very important for living beings are zinc (Zn) and manganese (Mn). They can either kill bacterial cells by breaking down their walls or stop critical metabolic processes [3]. When these metals are combined with organic ligands, they frequently perform better as stoppers and are easier for living things to use. As a result, they are a more environmentally friendly and long-lasting option than traditional treatments [2, 3]. In recent years, "green synthesis" has become the most popular method for creating bioactive inhibitors. Beta vulgaris beetroot juice, like other natural plant extracts, contains bioactive molecules such as betalains, polyphenols, and organic acids. These chemicals are natural chelators that combine with metal salts to form stable precipitates [4]. Beets are ideal for this plan because they have high antioxidant levels, degrade quickly, and are environmentally friendly. More research is being done on how to improve functional materials rather than how to extract inhibitors from natural fluids or make them more accessible. Most modern research focuses on creating complex ligands in the lab rather than employing simple, low-cost, and environmentally friendly methods [5]. We still don't know how the chemicals in beetroot extract react with simple transition salts like manganese(II) chloride ($MnCl_2$) and zinc(II) chloride ($ZnCl_2$) to form precipitates or how well they work, especially when it comes to killing bacteria. This study aims to address this issue by creating metal-based inhibitors using a rapid precipitation method. To create precipitates, we combine beetroot juice with water solutions of $ZnCl_2$ and $MnCl_2$. Then we check to see how well they stop bacteria from growing. This discovery leads to the creation of environmentally friendly antibacterial treatments that use the natural synergy between ligands from plants and transition

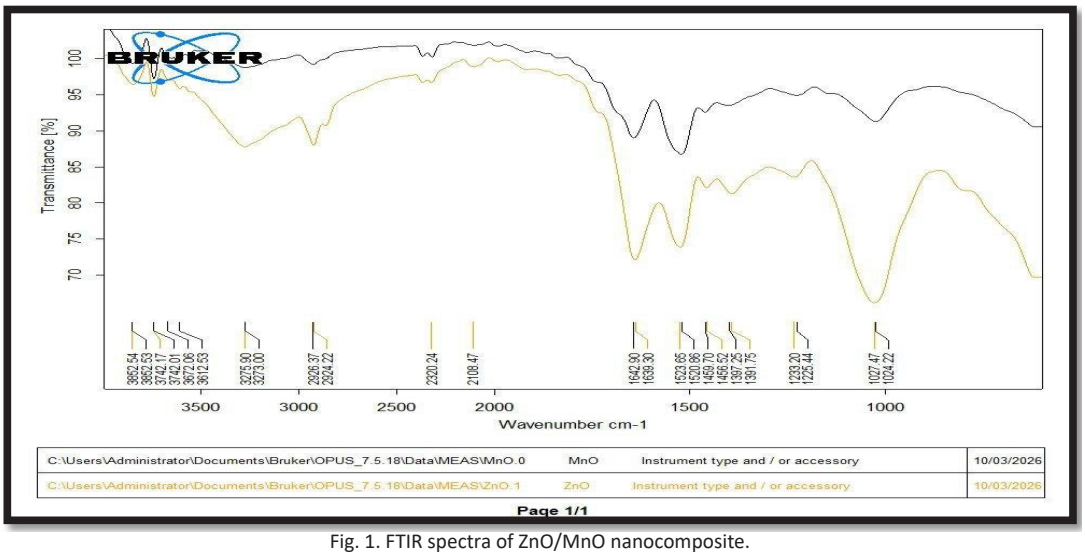

Fig. 1. FTIR spectra of ZnO/MnO nanocomposite.

Table 1. Weight Precentage of the Metal-Organic Complex Prepared Using Beetroot Juice.

Sample	Wt (gm)	Deionized Water (ml)	Beetroot juice(ml)
$ZnCl_2$(gm)	4.18	400	100
$MnCl_2$(gm)	7.2	400	100

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metals [6].

MATERIALS AND METHODS

The main minerals used were manganese chloride ($MnCl_2$, molar mass 197.91 g/mol) and zinc chloride ($ZnCl_2$, molar mass 136.30 g/mol). All of the chemicals were of analytical grade and came from Thomas Baker in India. All of the studies used deionized water without any extra steps to clean it. In a glass beaker, 4.18 g of $ZnCl_2$ was dissolved in 400 ml of deionized water. The solution was stirred with a magnet for roughly an hour to make sure it was all the same. After that, as shown in Table 1, 100 g of freshly squeezed beetroot juice is added to the solution. The second mineral-organic complex was synthesized by dissolving 7.2 g of $MnCl_2$ in 400 ml of deionized water under magnetic stirring for approximately one hour until fully dissolved, followed by the incorporation of 100 ml of beet juice extract with ongoing stirring, as detailed in Table 1.

The reaction mixtures were shielded with aluminum foil and maintained at room temperature for 48 hours. Upon completion of the reaction period, the resultant sediment was isolated using centrifugation at 4000 r/min for 20 minutes, followed by three washes with deionized water to eliminate unreacted materials and residual contaminants. The resultant sediment was transferred to petri dishes and dehydrated in an electric oven at 550°C for approximately two hours to yield a solid nanopowder. Surface morphology was examined with a field emission scanning electron microscope (FESEM, Tescan MIRA3, Czech Republic), and elemental composition, weight, and atomic ratios were determined with energy-dispersive X-ray spectroscopy (EDX, Oxford Instruments, UK) and a scanning electron microscope (SEM). The hydrodynamic volume distribution of synthesized nanoparticles was measured with the Zetasizer Nano ZS apparatus (Malvern Panalytical Company, UK), which uses dynamic light scattering (DLS) technology. The antibacterial activity of the formulated compounds was evaluated against Staphylococcus aureus and Escherichia coli. The implant dishes were incubated at 37°C for 48 hours, and the inhibitory zone widths were measured in millimeters to determine antigenic activity.

RESULTS AND DISCUSSION

The image depicts an FT-IR spectrum comparing

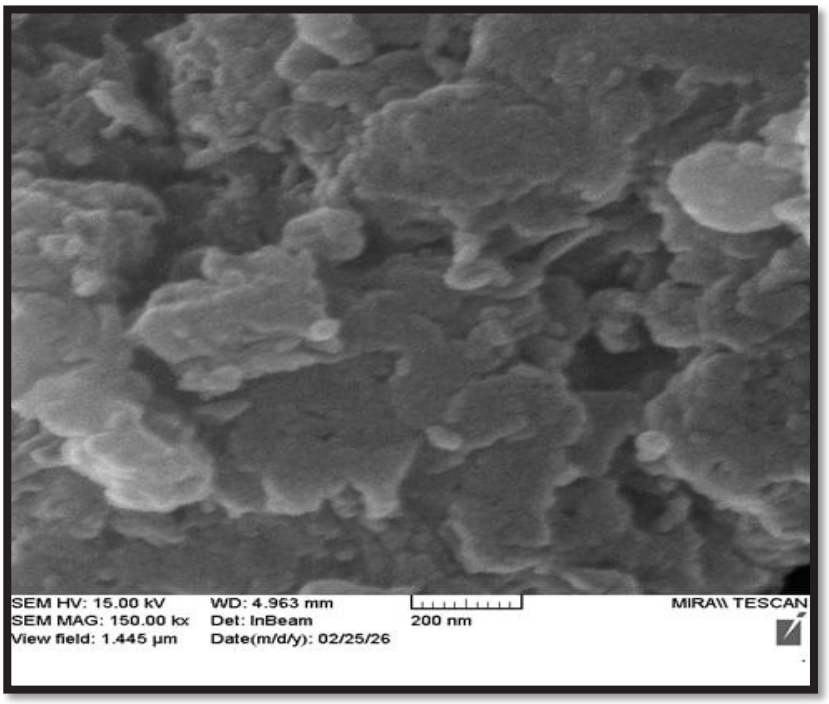

Fig. 2. FE-SEM images of different concentrations of ZnO composites Beet juice.

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manganese oxide (MnO) and zinc oxide. This study employs a basic spectroscopic technique to characterize chemical bonds and lattice vibrations in metal oxides, as well as to identify adsorbed surface groups on nanomaterials. In the region of elevated wave numbers (3600-3200 cm^{-1}), a broad absorption band emerges due to the tensile vibrations of hydroxyl aggregates (O-H), indicating the presence of water molecules or adsorbed groups on the nanoparticle surface. This is a common occurrence in metal oxides with high surface area and reactivity [7]. Moderately weak peaks are seen around 2926–2924 cm^{-1}.These are usually caused by the tensile vibrations of C-H aggregates that are left over from the organic chemicals or feedstocks used in the manufacturing process. In the mid-infrared spectrum, specifically at 1640 cm^{-1}, absorption peaks appear that are due to the bending vibrations of adsorbed water molecules (H–O–H bending vibration). This means that there is physically adsorbed water on the oxide surfaces. There are extra peaks in the range of 1500–1200 cm^{-1}, which could be due to C-O bond vibrations, leftover nitrate or carbonate groups from the starting materials, or the surface interacting with carbon dioxide in the air. This has been seen in many studies that use FT-IR to describe nanooxides [8]. The spectral footprint (below 1000 cm^{-1}) shows big peaks that show metal oxide production. This is because this area corresponds to the lattice vibrations of metal–oxygen connections. Unique absorption peaks appear in the 600–400 cm^{-1} range for ZnO because the Zn–O bond in the wurtzite hexagonal crystal lattice vibrates in a way that makes them. These peaks are signs that zinc oxide nanoparticles are forming. Many recent studies have shown that the Zn–O vibration range usually happens between 400 and 600 cm^{-1}, which is clear proof of the oxide's crystalline structure [9, 10]. On the other hand, the MnO spectrum shows absorption bands in the lower part of the spectrum. This is because the Mn–O bond inside the crystal structure vibrates.

Field emission scanning electron microscopy (FE-SEM)

The field emission scanning electron microscopy (FESEM) picture of the nano-zinc oxide (ZnO) sample shows a clustered nanomorphological structure made up of agglomerates that are not perfectly formed and have a rough surface. The

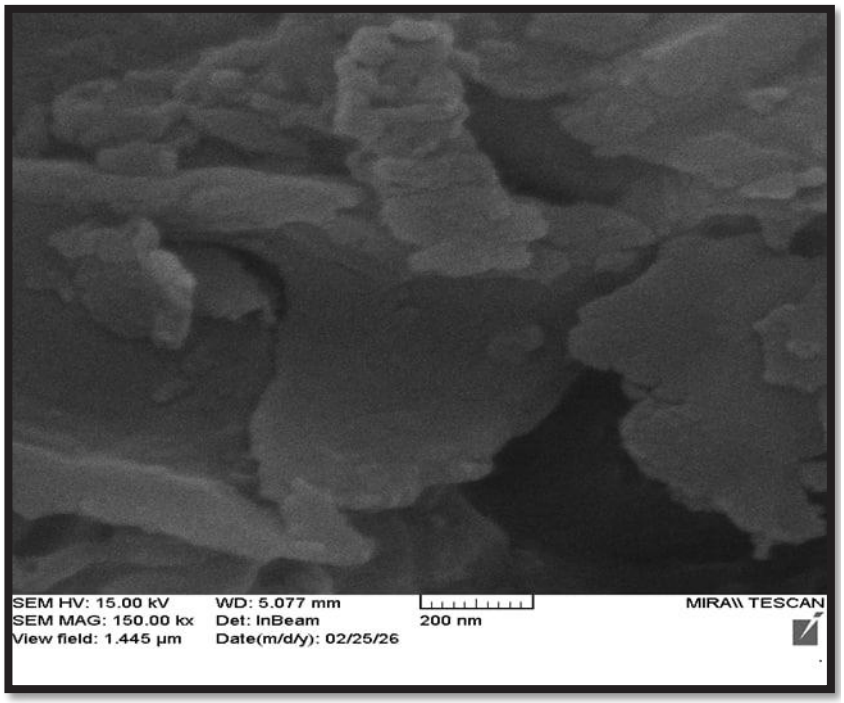

Fig. 3. FE-SEM images of different concentrations of MnO composites Beet juice.

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scale bar in the picture (500 nm) shows that the agglomerates are made up of tiny primary nanoparticles that are tightly packed and connected. This is because the nanoparticles have strong surface attraction forces that keep them together. The zinc nanoparticles in this sample are about 25 to 63 nanometers wide. The image shows some agglomeration, which is common in zinc oxide nanoparticles because of high surface energy and van der Waals forces. This causes particles to stick together to lower the system's free energy and make the structure more stable. The rough surface and granular agglomerates may increase the material's specific surface area, which is very important for ZnO nanocomposites used in catalysis and photonics [11, 12]. The FESEM study results suggest the formation of ZnO nanoparticles in the nanoscale range. These nanoparticles have a clustered structure and a large surface area. The morphological features are in line with recent studies on how to make nanoscale zinc oxide using different chemical methods [13].

Field emission scanning electron microscopy (FE-SEM)

The field emission scanning electron microscopy (FESEM) image of the manganese oxide (MnO_2) sample shows a nanostructure that looks like a lot of plates or particles stacked on top of each other to produce quasi-hexagonal nanoclusters. The scale in the figure (200 nm) shows that the nanoparticles or nanosheets are between 20 and 77 nanometers in size. This shows that the synthesis process worked and generated a substance that is quite small. This range of volumes reveals that small primary nuclei developed first. Then, because the nanometal oxides had a lot of surface energy, the particles grew and stayed together. A lot of the time, this happens with MnO_2 materials produced by chemical or hydrothermal methods [14]. The picture reveals a clustered shape, which means that a hierarchical nanostructure has evolved. Small primary units have come together to produce a greater secondary structure. This structural arrangement is recognized for making the surface area more effective and improving the movement of ions and electrons. Recent studies have shown that manganese oxide plates or nanoparticles within a range of less than 100 nm possess high surface activity due to the increased ratio of surface atoms and crystalline defects, which positively impacts

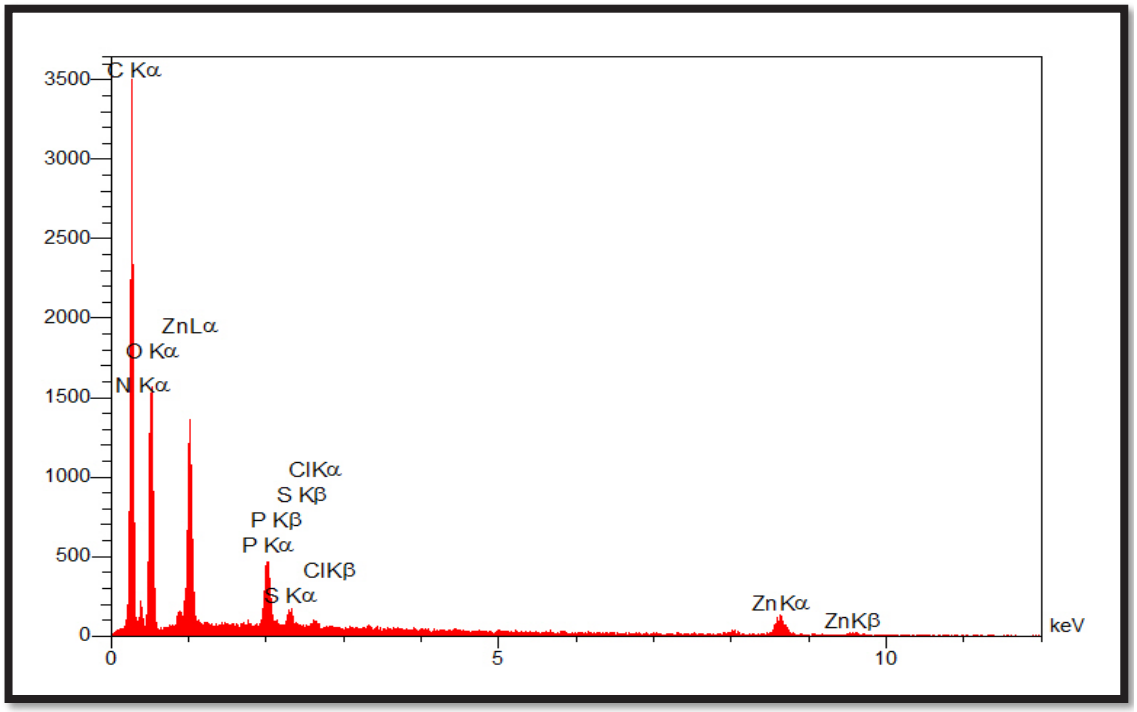

Fig. 4. EDS results for the different chemical composition of the precipitated Metal-organic complex.

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electrochemical and catalytic applications [15,16]. The intercalation of nanosheets or clusters can create interstitial spaces and fine pores that contribute to improved mass transfer within the material, which is an important factor in the performance of MnO_2 in supercapacitors and catalytic systems [17]. Based on that, the results of the FESEM examination indicate that the sample possesses nanoparticles with a size of 20–77 nm and a plate-like agglomerated morphology, which is a structure suitable for increasing the surface area and the number of active sites, thereby supporting its potential use in advanced applications of nanomaterials.

The EDS spectrum shown in the picture shows how the chemical elements are spread out in the sample that was analyzed. The different spectral peaks show the different emission lines of the elements in the measured energy range in keV. The spectrum shows multiple strong peaks that are caused by different basic parts. At a low energy level of about 0.27 keV, a strong peak of great intensity appears. This peak is due to carbon (C Kα) and is the most important peak in the spectrum. It shows that there is a lot of carbon in the sample. People usually say that carbon is there because of the organic makeup of the covalent compound or because of the carbon substrate or adhesive tape that the electron microscope uses. There are also extra peaks at low energies that correspond to oxygen (O Kα) and nitrogen (N Kα). This means that the sample's structure has functional groups that contain nitrogen and oxygen. This is primarily about how organic ligands function in molecules with covalent bonds, such as amines, imines, and carboxylic groups. The medium energy range (about 2-3 keV) shows distinct peaks for chlorine (Cl Kα and Cl Kβ), phosphorus (P Kα and P Kβ), and sulfur (S Kα and S Kβ). The presence of these elements indicates that the ligand or sample may contain partner ions or functional groups made of chlorine, sulfur, and phosphorus. The zinc element (Zn Kα and Zn Kβ) appears as two peaks in the high-energy spectra, one at 8.6 keV and the other at 9.6 keV. These peaks help us detect zinc ions in the sample. The peaks show that zinc is the metal core of the harmonic compound that was studied. This is consistent with the design of many harmonic compounds, including zinc, as it is stable in complexes and possesses favorable electrical properties. The relative strength of the peaks shows that the peaks of the light elements (C, O,

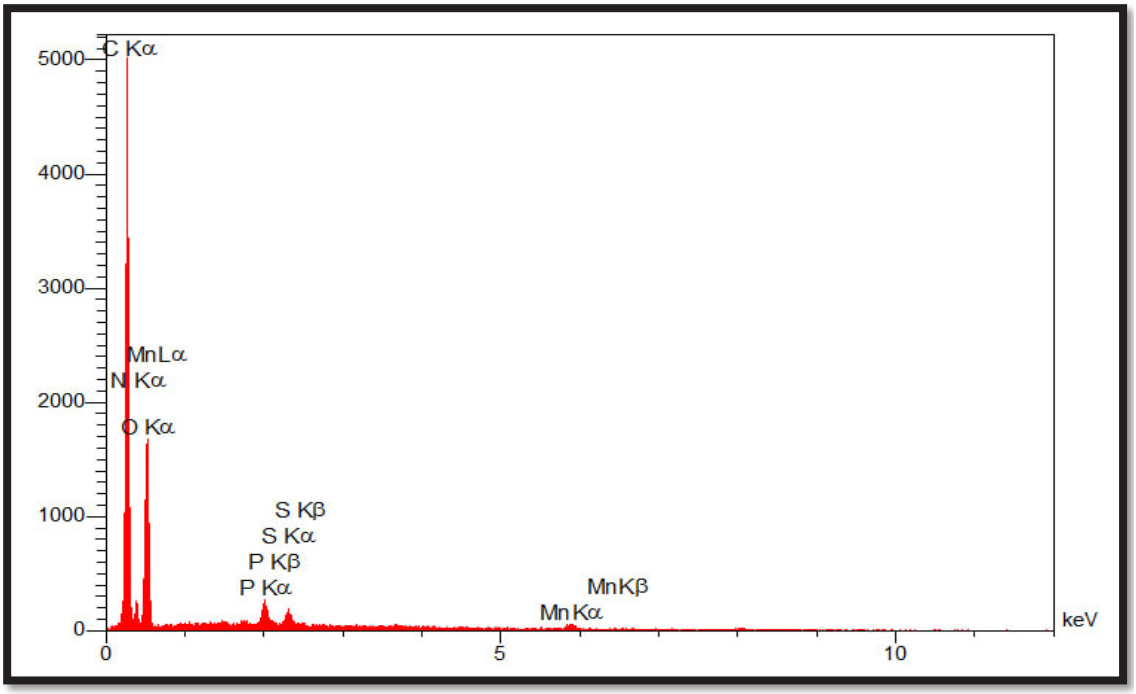

Fig. 5. EDS results for the different chemical composition of the precipitated Metal-organic complex.

N) are quite strong since they are very sensitive in this energy range. The peaks of zinc, on the other hand, are not as prominent but are still clear and distinct. This reveals that it is part of the sample's chemical makeup. The EDS analysis demonstrates that the coordination compound has its main parts: zinc as the metal center; carbon, nitrogen, and oxygen that make up the ligand's organic structure; and chlorine.

The energy-dispersive X-ray spectroscopy (EDS) spectrum makes it very clear which elements are in the sample. You can easily see the peaks for carbon (C), manganese (Mn), phosphorus (P), nitrogen (N), and sulfur (S). This means that a chemical system is made up of many different parts. This could be because a coordination complex was made with a manganese metal core and organic or inorganic ligands that have donor atoms such as N, O, S, and P. There is a strong carbon peak at a low energy level of about 0.27 keV. This means that there is an organic framework inside the structure. Many metal-organic complexes have this in common: they have organic ligands like thiols, phosphates, or heterocycles [18]. The peaks for N Kα and O Kα are low, at 0.39 and 0.52 keV. This means that there are places where pairs of free electrons can move electrons to the center of the metal. This discovery fits with what we already know about coordination chemistry, which says that nitrogen and oxygen atoms are strong donors that can make coordination bonds with transition metals [19]. The phosphorus (P Kα and P Kβ) and sulfur (S Kα and S Kβ) peaks show that the ligand has thiol/thioether or phosphate groups in it. These groups are known for having strong interactions with coordination, which makes transition metals

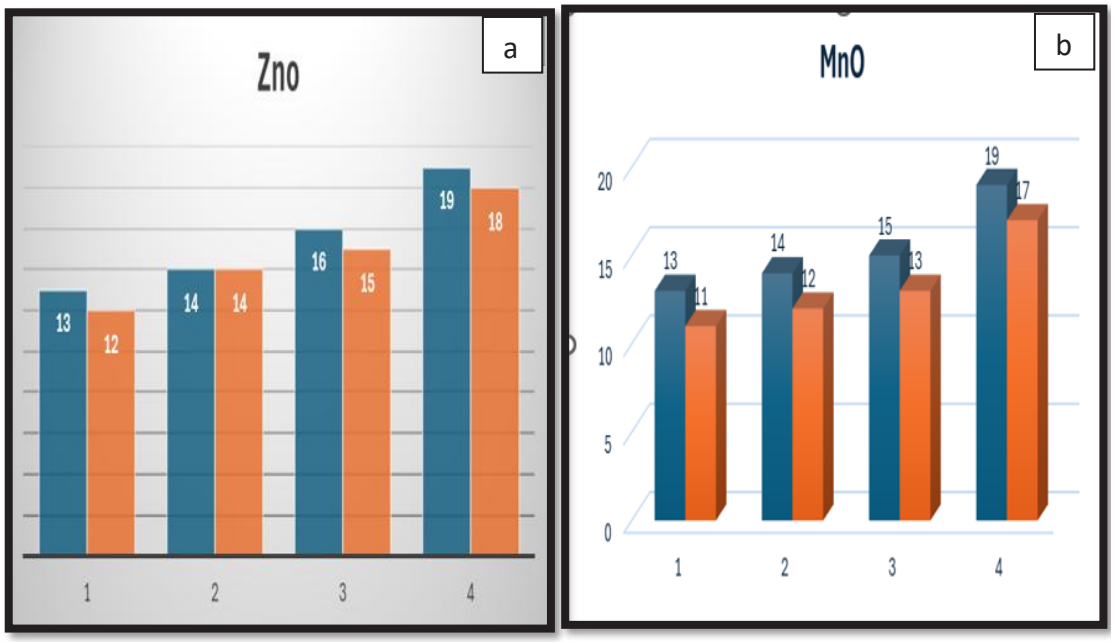

Fig. 6. Antibacterial activity of (Zn and Mn) against E.*coli* and S. *aureus* with different concentrations (125,250,500 and 1000 µg/mL.

Table 2. Concentration-Dependent Antibacterial Activity of Zn and Mn against E. coli and S. aureus (125–1000 µg/mL).

Bacteria	Symbol	Control	125	250	500	1000
Staph	Zn	0	R	13	14	16
E.coli	Zn	0	R	12	14	15
Staph	Mn	0	R	13	14	15
E.coli	Mn	0	R	11	12	13

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and their derivatives more stable. The manganese peaks Mn Kα (about 5.9 keV) and Mn Kβ (about 6.4 keV) in the spectrum show that the sample has the main metal element. This means that manganese is the metal that keeps the coordination framework together. The peaks that show up in an EDS analysis show that manganese has some spectral properties. The fact that they are stronger than lighter elements shows that there is less manganese than organic matter. This is what you would expect in metal-organic complexes with a high ligand-to-metal center ratio [20]. The peaks in the low-energy spectrum show that there are a lot of light parts in the mix. This is like how organic or semi-organic ligands work. The fact that there are no peaks for other metallic elements supports the idea that the metallic phase is mostly pure. This means that there aren't any big metal impurities at the device's sensitivity thresholds. Energy-dispersive X-ray spectroscopy makes it very clear that a coordination molecule was made with manganese and multi-site donor ligands (N, O, S, P). This fits with what we already know about how metal-organic complexes with manganese are made. These complexes are important for catalysts, advanced functional materials, and biological materials.

Antibacterial

The results presented in Table 2 indicate a clear inhibitory effect on bacterial growth when using samples containing zinc (Zn) and manganese (Mn), as the control group did not show any inhibition zone (0 mm), indicating that the recorded inhibition in the other treatments is directly due to the effect of these elements. It also appears that the low concentration (125) did not show antibacterial activity as the bacteria were recorded in a resistant state (R), while the inhibitory effect began to appear at higher concentrations (250, 500, 1000) with a gradual increase in the diameters of the inhibition zones. The samples containing zinc recorded inhibition zones ranging from 13–16 mm against Staphylococcus aureus bacteria, and between 12–15 mm against Escherichia coli bacteria. Meanwhile, the manganese samples also showed antibacterial activity but to a lesser extent, with inhibition zones ranging from 13–15 mm for Staphylococcus aureus and 11–13 mm for Escherichia coli.

From a taxonomic perspective, Staphylococcus aureus is classified as a Gram-positive bacterium, while Escherichia coli is classified as a Gram-negative bacterium. The difference in sensitivity between these two types is often attributed to the differences in the structure of the bacterial cell wall. Gram-positive bacteria have a thick layer of peptidoglycan but lack an outer membrane, while Gram-negative bacteria have an outer membrane rich in lipopolysaccharides that acts as an additional barrier limiting the permeability of certain antimicrobial agents [21]. Therefore, Gram-positive bacteria are often more sensitive to certain antibacterial agents compared to Gram-negative bacteria.

The antibacterial activity observed in this study can also be explained by known metal or metal nanoparticle effects, particularly zinc compounds, which cause membrane damage and increase permeability while also producing reactive oxygen species (ROS) that induce oxidative stress within the bacterial cell, resulting in protein and DNA damage and disrupting metabolic processes required for cell survival. Zn^{+} ions disrupt enzymatic systems, leading to bacterial growth or cell death [23].

The gradual increase in the diameter of the inhibition zones with higher concentrations indicates that the antibacterial activity of these materials is dose-dependent, where higher concentrations lead to increased interaction between the metal particles and bacterial cells, thereby enhancing the growth inhibition effect [24]. Recent studies also confirm that metal nanoparticles, particularly zinc oxide nanoparticles (ZnO nanoparticles), are promising materials in combating pathogenic bacteria, including Staphylococcus aureus (Gram-positive) and Escherichia coli (Gram-negative), due to their ability to cause multiple damages to bacterial cells such as disrupting the cell membrane, inhibiting enzymes, and affecting genetic material [25].

Based on this, it can be concluded that the current results are consistent with recent studies indicating that mineral elements or the nanoparticles derived from them possess antibacterial activity against both Gram-positive bacteria such as Staphylococcus aureus and Gram-negative bacteria such as Escherichia coli, and that this activity increases with higher concentrations, making these materials promising candidates for medical and environmental applications in combating pathogenic bacteria, especially in light of the increasing problem of antibiotic resistance

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[21-23].

CONCLUSION

Using natural beetroot (Beta vulgaris) extract, this study successfully developed a green synthesis method for making metal oxide nanoparticles (ZnO and MnO) that is cost-effective, long-lasting, and good for the environment. The precipitation method used beetroot juice as a natural chelating agent to change zinc and manganese chloride precursors into stable metal oxide powders. FT-IR and FESEM structural and morphological characterizations showed that ZnO particles ranged in size from 25 to 63 nm and MnO particles ranged in size from 20 to 77 nm. This showed that separate Zn–O and Mn–O bonds had formed. The EDS analysis also showed that the coordination complex was made correctly because the plant extract made an organic framework and the elements were very pure. The nanocomposites that were made had a strong antibacterial effect that depended on the dose against both Gram-positive (S. aureus) and Gram-negative (E. coli) bacteria. The stronger inhibitory effect is because the metal ions and the bioactive compounds in beetroot extract, like betalains and polyphenols, work together. This green synthesis method is a good, safe, and effective way to make multifunctional antimicrobial agents that can be used in cutting-edge medical and environmental settings. It is especially useful for fighting antibiotic resistance around the world.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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