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The potential role and biological assessment of Cu doped manganese oxide nanoparticles

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Abstract

Nanoparticles have significant attention for potential applications in diverse fields like materials chemistry, medicine, environmental studies, agriculture, catalysis, information technology, biomedical sciences, optics, electronics, energy, and sensors. Recently, an alternative technique for biological evaluation of copper doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) nanoparticles has been developed using plants, bacteria, fungi, and algae. This study was aimed to assess the antibacterial, antifungal and antioxidant activity of Cu doped manganese oxide nanoparticles. The work was conducted in University of Okara laboratory. Disc diffusion and well diffusion methods were used to test the nanoparticles for antibacterial activity against *Escherichia coli, Staphylococcus aureus,* and *Bacillus subtilis* strains. The antifungal and antioxidant activity was also assessed. When combined with 0.1 mg/ml ciprofloxacin, Cu-doped manganese oxide nanoparticles exhibited typical inhibitory effects against E. coli and Bacillus subtilis, with zone of inhibition values of 0.05, 0.075, and 0.1 mg/ml. However, they showed no antibacterial activity against *Staphylococcus aureus*, unlike levofloxacin, which had varying inhibitory effects. The nanoparticles lack significant antibacterial properties. Antifungal testing revealed a slight inhibitory effect on *Fusarium equesiti* only for three days, with no impact on *Rhizopus Stolonifer*. Notably, the nanoparticles displayed superior antioxidant activity, achieving 99% at 0.08 µg/ml, surpassing ascorbic acid's 86%. Thus, nanoparticles may serve as potent antioxidants. To conclude, the synthesized Cu doped manganese oxide NPs can be used as a novel antibacterial, antifungal and antioxidant agent in agriculture.

Keywords: Nanoparticles, Copper, Manganese oxide, Antibacterial, Antifungal, Antioxidant

Introduction

Nanotechnology is a rapidly growing field with significant impact across industries including

pharmaceuticals, food, healthcare, chemicals, electronics, energy, cosmetics, space, and environmental sciences (1).With a soaring demand for nanotechnology-based products, this innovative discipline has the potential to greatly enhance human health and well-being (2). Nanotechnology focuses on developing and synthesizing diverse nanomaterials, which are particles sized from 1 to 100 nanometers. Their small size grants them unique properties distinct from bulk materials (3). Nanoparticles are classified into organic and inorganic types. Inorganic nanoparticles include magnetic (Co, Fe, Ni), semiconductor (ZnO, ZnS, CdS), and metal nanoparticles (Au, Ag, Cu, Al). Organic nanoparticles are carbon-based, such as fullerenes, quantum dots, and carbon nanotubes (4).

Biological entities provide a sustainable alternative for green synthesis of nanoparticles, avoiding toxic compounds and eliminating the need for external reducing, capping, and stabilizing agents. These methods work under moderate pH, pressure, and temperature conditions, simplifying nanoparticle formation (5). There have been recent events the significant increase in the utilize of antibiotics for the treatment of various bacterial infections (6). It is crucial to acknowledge that bacteria can develop antibiotic resistance (7). The rise of antibiotic resistance has resulted in a rise in the severity and fatality of bacterial infections. Antibiotic-resistant bacteria now claim more lives than cancer and diabetes combined (8). Developing new antibacterial agents is crucial for global public health. Emphasizing ecologically safe, recyclable, and cost-effective nanomaterials and production techniques is essential in this pursuit. (9). There has been a growing interest in metal and metal oxide-based nanoparticles as potential candidates for this purpose (10).

Copper and its derivatives are known for their strong antibacterial properties due to the release of copper ions, which weaken bacterial cell walls, induce oxidative stress, and cause genotoxic effects, leading to bacterial destruction(11). Doping enhances nanoparticles' properties for biomedical applications through physical, biological, and chemical techniques. Key methods include top-down (breaking down larger materials) and bottom-up (assembling smaller units). Techniques like sol-gel, arc discharge, laser treatment, and sonication are used, with sol-gel being favored for its simplicity and purity(12). Antibacterial tests revealed that Cu-doped MgO nanoparticles were especially effective against gram-positive S. aureus strains(13). Copper NPs have been observed to undergo rapid oxidation, leading to the formation of copper oxide, as described by (14).Manganese nanoparticles are eco-friendly catalysts due to their abundance, cost-effectiveness, and stability. With five unpaired d-electrons, they can exist in multiple oxidation states, enhancing oxygen mobility and storage during oxidation and improving catalytic performance (15). Given the challenges of contagious diseases and antibiotic resistance, nanoparticles are emerging as highly innovative and effective therapeutic agents in antimicrobial applications(16). Copper (Cu) and manganese (Mn) are essential microelements with key biomedical applications. They act as antioxidants for neurological disorders and are used in cancer treatment and imaging. Their roles are well-studied.(17). Copper nanoparticles (Cu-NPs), in contrast to conventional fungicides, have exhibited robust antifungal activity against phytopathogenic fungi (18). However, further investigation and assessment are required in this area due to the limited research available on the antifungal action of Cu-NPs against plant pathogenic fungi (19). The purpose of this study was to evaluate the antibacterial activity of Cu-doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) nanoparticles against *Bacillus subtilis* and *Escherichia coli*, also to assess the susceptibility and resistance patterns of different antibiotics against *Staphylococcus aureus*, and the antifungal activity against *Fusarium equesiti* and *Rhizopus stolonifer*, and determine their antioxidant activity.

Material and Methods

Study Area and Chemicals

This study was carried out at Okara University, Department of Zoology Management Research Laboratory. All experiments for synthesizing Cu-doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) nanoparticles used analytical-grade materials from the University of Okara, RenalaKhurd. *Staphylococcus aureus* cultures were sourced from Allama Iqbal Medical College, Lahore; *E. coli* and *Bacillus subtilis* from the University of Agriculture, Faisalabad. Fungal cultures and antioxidants, including ascorbic acid and DPPH, were obtained from the University of Okara, with additional nutrients and antibiotics provided by the University's Department of Zoology.

Apparatus and Equipment

The materials used included nutrient agar, autoclaved nutrient broth, weighing tools, distilled water, measuring cylinders, conical flasks, beakers, sterile petri plates, foil, non-absorbent cotton, parafilm tape, forceps, cotton swab sticks, incubator, autoclave, refrigerator, scissors, millimeter scale, falcon tubes, micropipettes (100-1000 µl), methanol, ethanol, transferring loops, a biosafety cabinet, and a vortex mixer..

Synthesis of Cu doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) NPs

A mixture of 1.896g of potassium permanganate ($KMnO_4$) and 0.90g of copper(II) nitrate trihydrate ($Cu(NO_3)_2$) in 100 mL of water was combined with 1% SDS and 20 mL of HCl. The solution was autoclaved at 180°C for 24 hours. After cooling, a dark powder, $Mn_{0.88}Cu_{0.16}O_2$, was filtered and washed. PXRD peaks were observed at 2θ (12.8, 18.7, 28.5, 34.2, 42.6, 52.6, 62.7, 76.4) with D (38.2±5). FTIR showed bands at 520 cm^{-1} (Mn-O), 1370, and 1656 cm^{-1}. Elemental analysis (EDX) revealed Mn 41.34%, Cu 8.08%, and O 50.02% (20).

Characterization of Cu doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) NPs

The behavior, bio-distribution, safety, and efficacy of nanoparticles are strongly influenced by their physicochemical properties. Therefore, it is crucial to thoroughly characterize Cu-doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) nanoparticles to assess their functional attributes. Various analytical techniques, including powder X-ray diffraction (PXRD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and Brunauer-Emmett-Teller (BET) analysis, are used to comprehensively understand their structure and properties (20).

Biochemical tests for confirmation of bacteria

Based on their characteristic morphological appearance, colonies were identified as *Staphylococcus aureus, Bacillus subtilis,* or *Escherichia coli* species using the catalase test. The catalase test was conducted using the agar slant method with a few drops of 3% hydrogen peroxide to identify Staphylococcus aureus, Bacillus subtilis, and Escherichia coli. Catalase-positive colonies produced immediate bubbles upon the addition of hydrogen peroxide. Blood agar was not used, as it naturally produces bubbles, potentially confounding the test results (21).

Antibacterial activity

The nutrient broth and nutrient agar were provided by the Department of Zoology Laboratory at the University of OkaraRenalaKhurd. For nutrient broth preparation, 13 grams of the broth were mixed with one liter of distilled water, heated while stirring until fully dissolved, and then autoclaved at 121°C for 15 minutes for sterilization. Similarly, 7 grams of nutrient agar were combined with 250 ml of distilled water, heated with stirring until dissolved, and also autoclaved at 121°C for 15 minutes to ensure sterility (22).

Antimicrobial susceptibility test

Antibacterial activity was evaluated using the disc diffusion method. Staphylococcus aureus was grown in nutrient agar, and Cu-doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) NPs were prepared in ethanol and water at concentrations of 0.02 to 0.1 mg/ml. Sterile 6mm discs were soaked with these solutions and placed on the agar plates alongside a 5µg Levofloxacin control. After 24-hour incubation at 37°C, the zones of inhibition were measured to assess antibacterial efficacy (23).

The antibacterial activity of Cu-doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) NPs was tested against Bacillus subtilis and E. coli using the well diffusion method. Bacteria were standardized to 0.5 McFarland. Nutrient agar was prepared, poured into petri dishes, and wells were made. Various concentrations of NPs (0.025 to 0.1 mg/ml) were added to the wells and incubated at 37°C for 24 hours. Inhibition zones were measured and compared to ciprofloxacin (1 mg/ml) as a control.

Test compound relative percentage inhibition = 100 × (X-Y)/ (Z-Y)

Where X represents the entire test compound's area of inhibition, Y represents the entire solvent's area of inhibition, and Z represents the entire reference drug's area of inhibition(24).

Antibiotic susceptibility test

The antibiotic susceptibility of the collected bacterial cultures was evaluated using the classic disc diffusion method (25). Colonies were transferred to a test tube with sterile nutrient broth, and nutrient agar plates were inoculated with the culture using a sterile swab. After drying, antibiotic discs were placed on the agar, spaced at least 15 mm apart, and pressed gently. The plates were incubated at 37°C for 18–24 hours. Zones of inhibition around the discs were measured to assess antibiotic susceptibility (26). According to the CLSI explanatory criteria, the size of the zone of inhibition is utilized to categorize the organism as susceptible, intermediate, or resistant to particular antibacterial treatments (27)**.**

Antifungal activity

The antifungal activity of Cu-doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) NPs was evaluated using the well diffusion method on potato dextrose agar. After preparing and sterilizing the agar medium, petri plates were inoculated with spore suspensions of Rhizopusstolonifer and Fusariumequesiti. Wells (6 mm diameter) were filled with 100 µl of nanoparticle solutions at concentrations of 0.025, 0.05, 0.075, and 0.1 mg/ml. The plates were incubated at 28°C for 3, 5, or 7 days. Antifungal activity was assessed by measuring the zone of inhibition around each well (28).

Antioxidant activity

The radical scavenging ability of Cu-doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) nanoparticles was assessed by their capacity to neutralize DPPH free radicals. Various NP concentrations (0.02 to 0.2 mg/ml) were mixed with DPPH solution and incubated in the dark for 30 minutes at room temperature. Ascorbic acid was used as a reference. The free radicalscavenging activity of Cu doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) NPs was expressed as a percentage of inhibition and calculated using the following formula, as proposed by (29).

DPPH radical scavenging activity (%) = (Pc - Ps) / Pc × 100

Where: Ps is the absorbance of the sample containing Cu doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) NPsor standard ascorbic acid mixed with DPPH radicals. Pc represents the absorbance of the control solution with DPPH radicals

and methanol. This study aims to compare the scavenging activity of Cu-doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) NPs with conventional ascorbic acid to evaluate their potential antioxidant properties and explore their implications for various applications.

Results

Characterization of Cu doped manganese oxide NPs

X-ray diffraction (PXRD) analysis of Cu-doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) nanoparticles (NPs) revealed that the predominant phase was -MnO_2, with minor components of CuO_3 and $MnCuO_4$. The patterns showed a consistent decrease in the intensity of the -MnO_2 peak as the concentration of Cu increased, which was associated with the emergence of the CuO_3 peak. The primary phase, -MnO_2, was identified as having a tetragonal structure and belonging to the $P4_2/mnm$ space group. These PXRD results confirmed the monocrystalline nature of the sample and supported the elemental composition as shown in Figure 1.

The dislocation density values indicated that the nanomaterials in series-VI had fewer defects, smaller crystallite sizes, and thus superior stability. The crystalline volume values suggested that copper ions were effectively incorporated into the lattice sites of the samples. However, the presence of copper ions in the -MnO_2 structure led to increased crystal strain and more significant crystal defects. Calculations of lattice parameters revealed lattice contraction, which contributed to the positive tensile microstrain values observed in the nanomaterials.

Fourier transforms infrared spectroscopy (FTIR) for Cu doped manganese oxide NPs

The Fourier Transform Infrared (FTIR) spectra of sample 1 exhibited all the characteristic signals of the samples, as shown in Figure 1. Within the range of 1000 to 500 cm^{-1}, distinct bending and stretching vibrations of individual metal and oxygen bonds were observed. The FTIR spectra confirmed these findings, demonstrating a high degree of structural symmetry through a clear and consistent pattern.

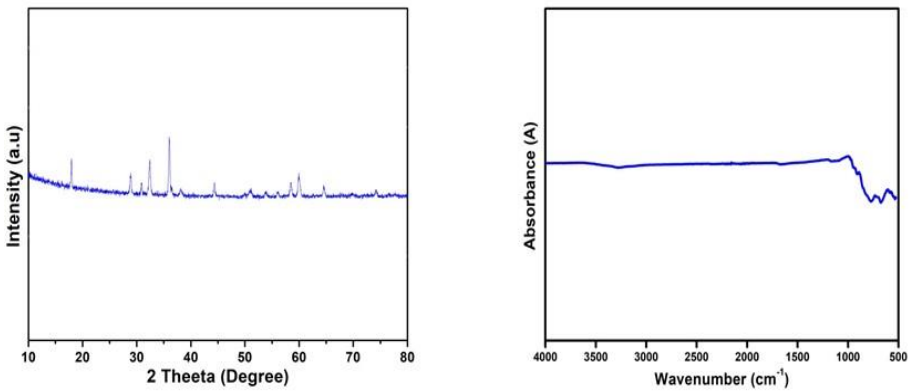

Figure 1: The PXRD patterns and FTIR spectra of nanoparticles

Brunauer–Emmett–Teller (BET) for Cu doped manganese oxide NPs

The specific surface area of the nanocatalysts was determined using the multipoint BET method, and the pore structure was analyzed using the DFT method. The nanocatalysts exhibited surface areas ranging from approximately 38 to 48 m^2/g. Notably, one sample had the highest surface area at 48.06 m^2/g, while another had a slightly lower surface area of 38.22 m^2/g. This indicates that the sample with 48.06 m^2/g has a larger surface area. Additionally, the pore volumes of 0.103 and 0.035 were relatively small, reflecting the inherent microporous nature of the nanomaterials. The consistent C values for all samples, being less than 100, suggest strong interactions between the solid adsorbate and the adsorbent, further emphasizing the porous characteristics of the materials.

Antibacterial assays

In the disc diffusion assay, Cu-doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) nanoparticles (NPs) were dissolved in 100 ml of pure ethanol and distilled water. Various sample concentrations were prepared (0.02 mg/ml, 0.04 mg/ml, 0.06 mg/ml, 0.08 mg/ml, and 0.1 mg/ml). These samples, along with a positive control containing 5 µg of levofloxacin, were tested against bacterial samples. Upon examination, the Cu-doped manganese oxide NPs did not produce any zone of inhibition, indicating a lack of antibacterial activity. In contrast, levofloxacin generated varying sizes of zones of inhibition, confirming its strong antibacterial properties. Based on these findings, it can be concluded that Cu-doped manganese oxide NPs do not exhibit antibacterial activity.

The antibacterial activity of Cu-doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) nanoparticles (NPs) was assessed at various concentrations (0.025 mg/ml, 0.05 mg/ml, 0.075 mg/ml, and 0.1 mg/ml) against selected bacterial strains. The results indicated that Cu-doped manganese oxide NPs were harmful to all tested bacterial strains at different doses. Ciprofloxacin was used as a standard drug. Among the bacterial strains examined, Cu-doped manganese oxide NPs exhibited the highest inhibitory activity against *E. coli*, followed by *Bacillus subtilis*, with zones of inhibition (ZOI) observed at concentrations of 0.05 mg/ml, 0.075 mg/ml, and 0.1 mg/ml, respectively, with the highest activity noted at 0.1 mg/ml in comparison to ciprofloxacin. Based on these findings, it can be inferred that Cu-doped manganese oxide NPs exhibit limited antibacterial action.

Antibiotic sensitivity pattern

Trimethoprim (W 5 µg), gentamicin (CN 10 µg), trimethoprim/sulfamethoxazole (SXT 25 µg), and streptomycin (S 10 µg) exhibited zones of inhibition against *Staphylococcus aureus*, while penicillin (P 10 U), amoxicillin (AML 10 µg), and ceftriaxone (CRO 30 µg) did not. These results indicate the susceptibility, intermediate susceptibility, and resistance of *Staphylococcus aureus* to various antibiotics.

The results reveal the susceptibility and resistance patterns of *Staphylococcus aureus* (Table 1). Trimethoprim (W 5 µg) and gentamicin (CN 10 µg) demonstrated intermediate results, while trimethoprim/sulfamethoxazole (SXT 25 µg) and streptomycin (S 10 µg) were effective in inhibiting *Staphylococcus aureus*. In contrast, penicillin (P 10 U), amoxicillin (AML 10 µg), and ceftriaxone (CRO 30 µg) showed resistance.

Table 1: Antibiotic resistance and susceptibility disc diffusion chart (*Staphylococcus aureus*)

Antibiotics	**Symbol**	**Concentration**	**Susceptible**	**Intermediate**	**Resistance**	**Zone of Inhibition**	**Results**

						in mm	
Trimethoprim	W	5ug	≥16	11-15	≤10	13	Intermediate
Streptomycin	S	10ug	≥15	12-14	≤11	18	Susceptible
Gentamicin	CN	10ug	≥15	13-14	≤12	14	Intermediate
Trimethoprim/ sulfamethoxazole	SXT	25ug	≥16	11-15	≤10	17	Susceptible
Amoxycillin	AML	10ug	≥20		≤19	0	Resistance
Penicillin	P	10U	≥29		≤28	0	Resistance
Ceftriaxone	CRO	30ug	≥21	14-20	≤13	0	Resistance

Antifungal activity of Cu doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) NPs

Cu-doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) nanoparticles (NPs) at various concentrations (0.025 mg/ml, 0.05 mg/ml, 0.075 mg/ml, and 0.1 mg/ml) were tested to evaluate their inhibitory activity against fungi over time. The results indicate that Cu-doped manganese oxide NPs have a slight inhibitory effect on the fungus *Fusarium equiseti* during the first three days. However, these NPs showed no inhibitory effect on the growth of the fungus *Rhizopus stolonifer* on days 3, 5, and 7. The maximum zone of inhibition was observed at a concentration of 0.05 mg/ml of Cu-doped manganese oxide NPs against *Fusarium equiseti*, measuring 1.2 mm on the third day. However, by the 5th and 7th days, the growth of *Fusarium equiseti* had completely covered the wells containing Cu-doped manganese oxide NPs. These results suggest that the inhibitory effect of Cu-doped manganese oxide NPs on *Fusarium equiseti* is only evident up to the 3rd day, after which no further inhibition is observed on days 5 and 7.

Antioxidant assays

The study results demonstrate that both ascorbic acid and Cu-doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) nanoparticles (NPs) exhibit antioxidant activity. Ascorbic acid was used as a reference, and various concentrations of Cu-doped manganese oxide NPs (0.02, 0.04, 0.06, 0.08, 0.1, 0.15, and 0.2 µg/ml) were tested. At the same concentration, ascorbic acid showed 86% antioxidant activity. In comparison, Cu-doped manganese oxide NPs exhibited even higher antioxidant activity, reaching a maximum of 99% at a concentration of 0.08 µg/ml. Even at a slightly higher concentration of 0.1 µg/ml, the antioxidant activity remained substantial at 91%. These findings suggest that Cu-doped manganese oxide NPs possess greater antioxidant activity than ascorbic acid, potentially making them more effective antioxidants for various applications (Figure 2).

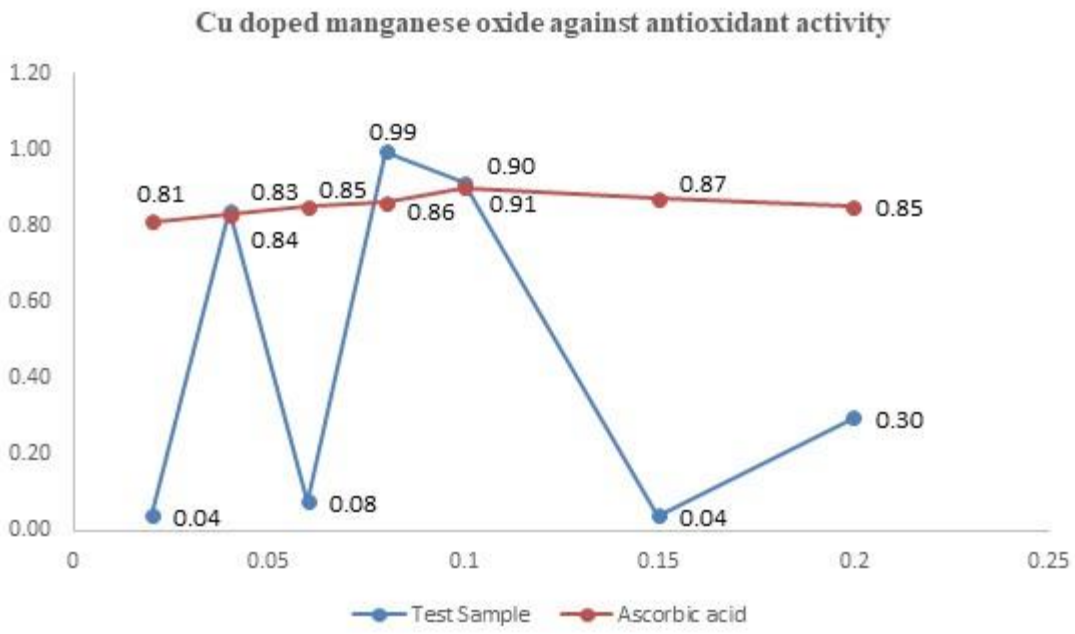

Figure 2: The antioxidant activity

Discussion

In this study, the biological evaluation of Cu doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) NPs was investigated. The antioxidant, antifungal, and antibacterial properties of the Cu doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) NPs were to be assessed. The gram-positive bacterial pathogens *Staphylococcus aureus*, *E. coli*, and *Bacillus subtilis* were more effectively neutralised by the Cu doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) NPs than by the rest of the examined microorganisms. This suggests that the nanoparticles may contain antibacterial compounds that are effective against most of the tested microorganisms.

Additionally, the well diffusion method was used to test the Cu doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) NPs antifungal efficacy against *Rhizopusstolonifer* and *Fusariumequesiti*, respectively. According to the study, ascorbic acid and Cu doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) NPs both have antioxidant properties.Using specific microorganisms, the antibacterial activity of Cu doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) NPswas designed. The NPs displayed some antibacterial activity as an inhibitory zone against well-known microorganisms using the diffusion approach. These findings demonstrated the amazing potential of Cu doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) NPs as an antibacterial agent in the treatment of bacterial infectious illnesses. Additionally, we have established the Cu doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) NPs minimum inhibitory concentrations for *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus*.

The test was conducted utilizing various microbe*Bacillus subtilis*and *Escherichia coli* concentrations with standard ciprofloxacin in the range of 0.05 mg/ml, 0.075 mg/ml, and 0.1 mg/ml. Similar results were obtained by Premanathan, Karthikeyan (30); However, it should also be noted that in the case of ZnO nanoparticles, the inhibition-zone diameters of Gram-negative bacterial strains of *E. coli* and *P. aeruginosa* were 24% and 16% lower than those of Gram-positive bacterial strains of *B. subtilis*and *S. aureus*. The zone diameters of identical Gram-

negative bacterial strains of *E. coli* and *P. aeruginosa* on CuO NPs were 28% and 33% smaller than those of the Gram-positive bacterial strains *B. subtilis* and *S. aureus*, respectively.

This observation might potentially be a sign that Gram-negative bacterial strains are more resistant to or tolerant of such nanomaterials than Gram-positive bacterial strains. Premanathan, Karthikeyan (30)showed that the nanoparticle ZnO impact is greater over Gram-positive isolates than Gram-negative bacterial strains; our findings support their findings. According to Mohammed, Mubark (31)study CuO NPs antibacterial activity was evaluated against different bacterial strains (including *E. coli* and *P. aeruginosa*). CuO NPs showed outstanding antibacterial activity against a variety of bacteria when tested for antibacterial activity against different bacteria using a well diffusion experiment.The size of the inhibitory zone represents how susceptible the microorganisms are.At a temperature of 200°C, the tiniest CuO nanoparticles (particle size 15.3 nm) were created.

According to Panáček, Kvítek (32)observation Although *S. aureus* and *E. coli* both show greater sensitivity to silver nanoparticles than to nanoparticles of copper, the difference is smaller for *S. aureus* than for the bacterium *E. coli*. Additionally, no strain preference was found and all *S. aureus* strains showed the same sensitive to copper and silver nanoparticles. The same outcomes showing no strain specificity were reported. According to Behera and Debata (33) studied the lowest level of antibacterial activity was seen in silver nanoparticles, which created no zones of inhibition against any of the *E. coli* strains while producing tiny zones of inhibition against *E. coli* strain 1-3 in 20% silver nanoparticles. Against *S. epidermidis* A37, 20% silver provided the highest average zone of inhibition (11.6 0.5 mm). Silver has been successfully applied in a number of biomedical applications.

In the current study, we discovered Cu doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) NPs utilized to combat the fungi *Rhizopusstolonifer* and *Fusariumequesiti*. The least amount of Cu doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) NPs, 0.05 mg/ml, was shown to have the greatest zone of inhibition against *Fusariumequesiti* (1.2mm).These findings demonstrated the exceptional potential of Cu doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) NPs as an antifungal agent for the treatment of infectious fungal infections. Similar results were obtained by Usman, Zowalaty (34) in compared to other samples, the CuNPs sample with the smallest particle (53 nm) displayed the best antifungal activity. Due to its smallest size, CuNPs with the smallest particle (53 nm) exhibit greater growth inhibition against *F. oxysporum* and *P. capsici*. This is because the smallest size is easily able to permeate cell membranes through its surface.

As a result, compared to other samples, CuNPs with smaller particle sizes exhibit the highest growth inhibitory activity. According to Ingle, Duran (35) observation in their trial, all of the plant pathogenic fungi were active against copper nanoparticles. Due to their numerous beneficial properties including antibacterial, antioxidant, anticancer, and antiviral ones copper nanoparticles have enormous potential in the field of biomedicine. According to Mahdizadeh, Safaie (36)depending on the *Fusarium* strains, AgNPs and silver ions have different fungistatic activities. Compared to the *F. avenaceum* strain, the *F. equiseti* strain shown higher sensitivity to the silver compounds. The various resistance mechanisms of the studied fungus may be the cause of the observed variations in responsiveness to AgNPs between strains.

According to Pariona, Mtz-Enriquez (37)high doses of CuONPs, 200 and 400 ppm, increased the suppression of *F. equiseti* growth. It should be emphasized that the fungicide potential of CuONPs is dependent on both its

concentrations and the previously stated fungi. According to Brahmanwade, Shende (38) studied *Fusarium* species, including *F. oxysporum*, *F. equiseti*, and *F. culmorum*, which are the most prevalent phytopathogens in Central India, have been confirmed to be resistant to the application of CuONPs as a fungicide. According to Viet, Nguyen (39) observation development of *F. equiseti* is inhibited. It was noted that *Fusarium sp*. did not increase with increasing incubation period from 3 to 9 days at 450 ppm CuNPs at varying concentrations of CuONPs at days 5 and 8. Ascorbic acid and Cu doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) NPs both appear to have antioxidant activity, according to the study.

Similar results were obtained by Chung, Abdul Rahuman (40); according to him, Using the free-radical complex diphenylpicrylhydrazyl, it is possible to assess the capacity of NPs to scavenge free radicals. The DPPH assay was used to examine the antioxidant activity of bio-synthesized copper NPs. The concentration of Cu NPs was raised, which boosted the DPPH scavenging activity.Similar results were obtained by Sridhar and Charles (41) according to him,It is quick, easy, and affordable to test the antioxidant properties of compounds using the free radical DPPH technique, which is frequently used to assess their capacity to act as independent-radical scavengers and hydrogen providers.

The DPPH test depends on DPPH, a stabilized free radical, being eliminated. In fact, DPPH is a stable free-radical molecule that has a dark color and crystalline structure. It is a recognized antioxidant and radical test in particular. The DPPH radical exhibits a deep purple hue in solution before being reduced and turned into DPPH-H, but after being reduced and changed into DPPH-H; it becomes colorless or light yellow. Same results were found by RajeshKumar and Rinitha (42) the stable free reagent DPPH has been widely used to assess reducing compounds and to investigate a component's capacity to scavenge free radicals. Copper nanoparticles ability to scavenge free radicals is quite similar to that of ascorbic acid in general. These findings demonstrated the amazing potential of Cu doped manganese oxide ($Mn_{0.88}Cu_{0.16}O_2$) NPs as an antioxidant in the treatment of oxidant infectious illnesses.

Conclusion

To conclude, the Cu-doped manganese oxide nanoparticles were evaluated for antibacterial, antifungal, and antioxidant activities. While they showed some inhibitory effects against E. coli and Bacillus subtilis when combined with ciprofloxacin, they were ineffective against Staphylococcus aureus. The nanoparticles exhibited minimal antifungal activity, affecting only Fusarium equiseti slightly. However, they demonstrated superior antioxidant activity, achieving 99% at 0.08 µg/ml compared to ascorbic acid’s 86%. Overall, these nanoparticles show promise as effective antioxidants and may have potential applications in agriculture

Statements and Declarations

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Competing interest

None

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None

Conflict of interest

None

Author's contribution

All authors contributed equally in the manuscript.

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