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Green Synthesis, Spectroscopic Characterization, and Structure–Activity Relationship of Novel Manganese–Benzamide Complexes with Antibacterial Activities

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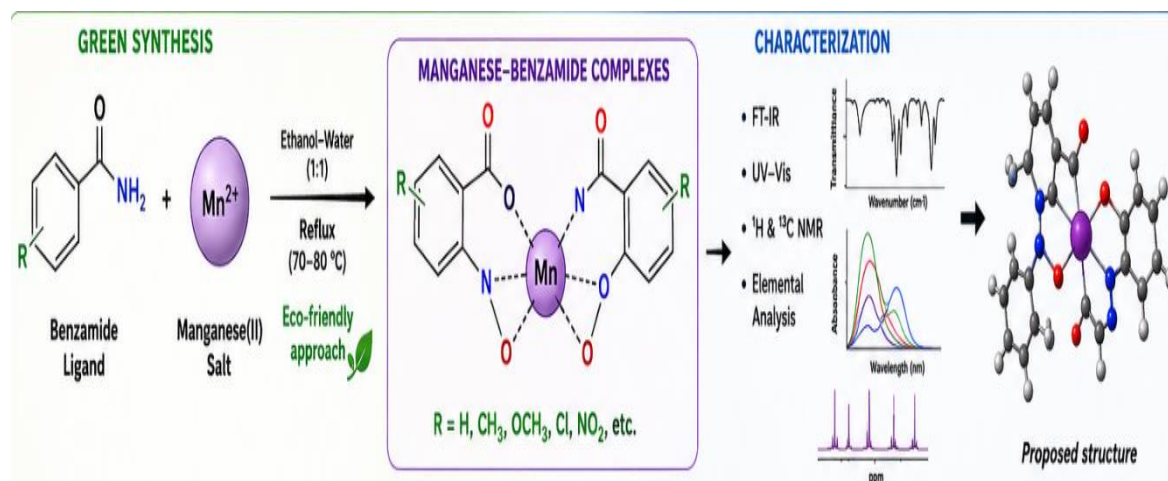
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ABSTRACT

GRAPHICAL ABSTRACT



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ABSTRACT

The development of novel metal-based antimicrobial agents has emerged as an effective strategy to combat the growing challenge of antimicrobial resistance and biofilm-associated infections. In the present study, a series of novel manganese–benzamide coordination complexes were synthesized through an environmentally friendly synthetic approach and comprehensively characterized using Fourier-transform infrared (FT-IR), ultraviolet–visible (UV–Vis), proton and carbon nuclear magnetic resonance (^1H and ^{13}C NMR), and elemental analyses to confirm their molecular structures and coordination behavior. The antibacterial potential of the synthesized complexes was systematically evaluated against representative Gram-positive and Gram-negative bacterial pathogens using multiple microbiological assays, including biofilm inhibition, time-kill kinetics, minimum bactericidal concentration (MBC), and shake-flask quantitative bacterial reduction methods. The synthesized manganese complexes demonstrated enhanced antibacterial efficacy compared with their corresponding free ligands, suggesting that metal coordination significantly improved their biological performance. The complexes effectively inhibited bacterial biofilm formation, exhibited concentration- and time-dependent bactericidal activity, and produced substantial reductions in viable bacterial populations under dynamic culture conditions. The improved biological activity is attributed to increased lipophilicity, enhanced membrane permeability, and synergistic interactions between the manganese ion and the benzamide ligand following chelation. Collectively, the findings demonstrate that manganese coordination considerably enhances the antimicrobial properties of benzamide derivatives and highlights the potential of these coordination compounds as promising candidates for the development of next-generation topical antibacterial agents for the prevention and management of bacterial and post-surgical wound infections. Further mechanistic investigations, cytocompatibility studies, and *In Vivo* evaluations are warranted to validate their clinical applicability.

Keywords: Manganese Complexes; Benzamide Ligands; Green Synthesis; Antibacterial Activity; Biofilm Inhibition; Minimum Bactericidal Concentration; Shake-Flask Assay; Transition Metal Complexes; Medicinal Inorganic Chemistry.

INTRODUCTION

Transition metal complexes have attracted considerable attention in medicinal chemistry owing to their diverse coordination geometries, tunable redox properties, and broad spectrum of biological activities. Unlike conventional organic drugs, metal complexes exhibit unique physicochemical characteristics that enable interactions with multiple biological targets, making them promising candidates for the treatment of cancer, bacterial infections, and other chronic diseases (Kozieł et al., 2024; Uddin et al., 2025). The clinical success of platinum-based anticancer drugs has further stimulated interest in developing alternative transition metal complexes with improved therapeutic efficacy and reduced systemic toxicity (Dimiza et al., 2024).

Among first-row transition metals, manganese has emerged as an attractive metal ion because of its low toxicity, environmental compatibility, and essential physiological functions. Manganese serves as a cofactor for numerous enzymes involved in

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antioxidant defense, cellular metabolism, and DNA synthesis. Furthermore, manganese complexes have demonstrated significant anticancer, antibacterial, antioxidant, and enzyme inhibitory activities, highlighting their potential as multifunctional therapeutic agents (Kozieł et al., 2024). Their biological activity is largely influenced by the nature of the coordinating ligands, which can alter the electronic properties, lipophilicity, stability, and cellular uptake of the resulting coordination compounds (Dimiza et al., 2024). Benzamide derivatives constitute an important class of bioactive molecules with diverse pharmacological properties, including antimicrobial, anticancer, anti-inflammatory, antiviral, and analgesic activities. The presence of amide functional groups provides excellent coordination sites for transition metal ions, facilitating the formation of stable metal complexes with enhanced biological performance (Uddin et al., 2025). According to chelation theory, coordination of ligands to metal ions reduces the polarity of the central metal atom while increasing the lipophilicity of the complex, thereby improving membrane permeability and enhancing interactions with intracellular biological targets (Kozieł et al., 2024).

The increasing prevalence of antimicrobial resistance and the continued global burden of cancer have intensified the search for novel therapeutic agents with improved efficacy and lower toxicity. Conventional antibiotics and chemotherapeutic drugs frequently suffer from multidrug resistance, severe adverse effects, and limited selectivity toward diseased cells, necessitating the development of alternative treatment strategies (World Health Organization [WHO], 2023). In this context, manganese coordination compounds represent a promising class of metal-based therapeutics because they exhibit multiple mechanisms of biological action and may overcome some limitations associated with traditional drugs (Kozieł et al., 2024). Green chemistry has also become an important consideration in the synthesis of medicinal compounds. The use of environmentally benign solvents such as water reduces hazardous waste generation and promotes sustainable synthetic methodologies without compromising reaction efficiency (Anastas & Zimmerman, 2020). Consequently, the development of manganese complexes through green synthetic approaches not only supports environmental sustainability but also contributes to the advancement of eco-friendly pharmaceutical research. In the present study, novel manganese–benzamide coordination complexes were synthesized using an environmentally friendly synthetic protocol and characterized by spectroscopic techniques. Their biological activities were subsequently evaluated to investigate the influence of manganese coordination on the therapeutic potential of benzamide ligands. The findings are expected to provide valuable insights into the design of new manganese-based coordination compounds for future medicinal applications.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and solvents were of analytical reagent (AR) grade and were used without further purification unless otherwise stated. Manganese(II) chloride tetrahydrate ($\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$), substituted benzoic acid derivatives, amines, ethanol, methanol, dimethyl sulfoxide (DMSO), sodium hydroxide, hydrochloric acid, and other reagents were purchased from commercially available suppliers. Deionized water was used

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throughout the study. Standard microbiological media, including Mueller–Hinton broth (MHB), Mueller–Hinton agar (MHA), and tryptic soy broth (TSB), were obtained from recognized commercial manufacturers.

Instrumentation

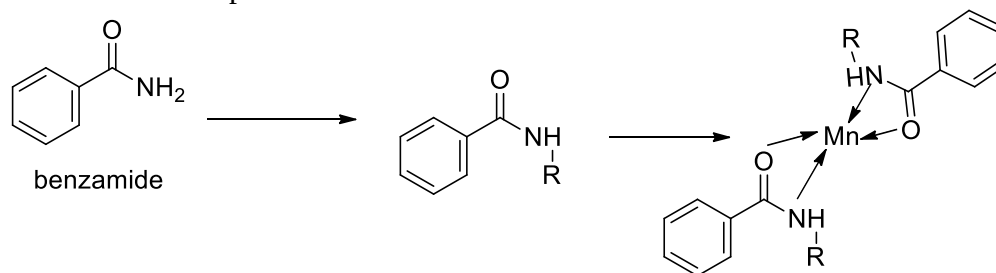
Fourier-transform infrared (FT-IR) spectra were recorded in the range of 4000–400 cm^{-1} using the KBr pellet method. Proton (^1H) and carbon-13 (^{13}C) nuclear magnetic resonance spectra were recorded using deuterated solvents with tetramethylsilane (TMS) as the internal reference. Ultraviolet–visible (UV–Vis) spectra were obtained using a double-beam UV–Vis spectrophotometer. Elemental analysis (C, H, and N) was performed to verify the composition of the synthesized compounds. Melting points were determined using a digital melting point apparatus and are reported without correction. Standard characterization procedures were performed according to established analytical protocols (Silverstein et al., 2014).

Synthesis of Benzamide Ligands

The benzamide ligands were synthesized by the condensation of the appropriate substituted benzoic acid derivatives with the corresponding amine under reflux conditions in ethanol. The reaction mixture was continuously stirred until completion, as monitored by thin-layer chromatography (TLC). The crude product was cooled to room temperature, filtered, washed with cold ethanol, and dried under vacuum. The purified ligand was obtained by recrystallization from ethanol and stored in a desiccator until further use.

Synthesis of Manganese Complexes

The manganese complexes were prepared by reacting the synthesized ligand with manganese(II) chloride in an appropriate molar ratio under continuous stirring in ethanol–water solution. The reaction mixture was refluxed for several hours until complete complexation was achieved. The resulting precipitate was filtered, washed several times with distilled water followed by ethanol to remove unreacted starting materials, and dried under reduced pressure. The synthesized complexes were preserved in airtight containers for subsequent characterization and biological evaluation. As explained the reaction scheme below:



Reaction Scheme

Purification and Percentage Yield

The synthesized ligands and manganese complexes were purified by recrystallization using appropriate solvent systems. Percentage yields were calculated using the

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following equation:

$$\text{Yield (\%)} = (\text{Experimental Yield} / \text{Theoretical Yield}) \times 100$$

The purity of the synthesized compounds was assessed using melting point determination and spectroscopic characterization.

Spectroscopic Characterization

a. FT-IR Analysis

FT-IR spectroscopy was employed to identify the characteristic functional groups and confirm coordination between manganese ions and ligand donor atoms. Characteristic absorption bands corresponding to amide carbonyl groups, aromatic C=C stretching, M–O, and M–N vibrations were carefully analyzed (Silverstein et al., 2014).

b. UV–Visible Spectroscopy

Electronic absorption spectra were recorded to investigate ligand-field transitions and charge-transfer bands associated with manganese coordination complexes. Spectral data were used to obtain preliminary information regarding coordination geometry.

Nuclear Magnetic Resonance (NMR)

^1H and ^{13}C NMR spectra of the synthesized ligands were recorded to confirm their molecular structures. Chemical shifts were expressed in parts per million (ppm) relative to tetramethylsilane (TMS). Because paramagnetic manganese complexes may produce broadened or shifted signals, NMR analysis primarily focused on the free ligands.

Elemental Analysis

Carbon, hydrogen, and nitrogen contents were determined using an elemental analyzer to verify the empirical composition of the synthesized ligands and complexes.

Bacterial Strains and Culture Conditions

Representative Gram-positive and Gram-negative bacterial strains were cultured using Mueller–Hinton broth under aerobic conditions at 37 °C. Fresh overnight cultures were adjusted to approximately 0.5 McFarland turbidity standard before antimicrobial testing. All microbiological procedures were carried out under aseptic laboratory conditions following standard microbiological guidelines (Clinical and Laboratory Standards Institute [CLSI], 2024).

Biofilm Inhibition Assay

Biofilm inhibition activity was determined using the crystal violet microtiter plate method. Briefly, bacterial suspensions were incubated with different concentrations of the synthesized compounds in sterile 96-well microplates. Following incubation, planktonic cells were removed, and the adhered biofilm was gently washed with phosphate-buffered saline (PBS). Biofilms were stained using crystal violet solution, excess stain was removed, and the bound dye was solubilized using ethanol. Biofilm inhibition was quantified by measuring absorbance at 570 nm using a microplate reader (Stepanović et al., 2007).

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Time-Kill Assay

Time-kill studies were performed to evaluate the bactericidal activity of the synthesized compounds over time. Standardized bacterial suspensions were exposed to test compounds at predetermined concentrations. Aliquots were collected at selected time intervals, serially diluted, plated onto Mueller–Hinton agar, and incubated at 37 °C. Colony-forming units (CFU/mL) were counted after incubation, and bacterial survival curves were constructed according to established procedures (CLSI, 2024).

Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) was determined following broth dilution susceptibility testing. After incubation with different concentrations of each compound, aliquots from wells showing no visible bacterial growth were inoculated onto fresh Mueller–Hinton agar plates and incubated for 24 h. The lowest concentration producing no visible bacterial colonies was recorded as the MBC (CLSI, 2024).

Shake-Flask Quantitative Bacterial Reduction Assay

Quantitative antibacterial activity was evaluated using the shake-flask method. Bacterial suspensions were incubated with the synthesized compounds under continuous shaking for a specified period. After incubation, surviving bacteria were enumerated by serial dilution and plate counting. The percentage reduction in bacterial population was calculated by comparing viable cell counts before and after treatment.

Statistical Analysis

All experiments were performed in triplicate, and data are presented as mean $\pm$ standard deviation (SD). Statistical analyses were conducted using GraphPad Prism or SPSS software. Differences among experimental groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A value of $p < 0.05$ was considered statistically significant (Motulsky, 2022).

RESULTS AND DISCUSSION

The successful synthesis of the manganese–benzamide complexes demonstrates the feasibility of employing transition metal coordination to modify the physicochemical and biological properties of benzamide-derived ligands. Transition metal complexes have attracted considerable attention in medicinal chemistry because their variable coordination geometries, redox behavior, and tunable ligand environments enable interactions with multiple biological targets, resulting in enhanced pharmacological activity (Kozieł et al., 2024).

Spectroscopic characterization confirmed the successful coordination of the ligands to the manganese(II) center. The FT-IR spectra showed noticeable changes in the characteristic stretching frequencies of the ligand functional groups compared with those of the free ligands, indicating the participation of donor atoms in metal coordination. Furthermore, the appearance of new absorption bands in the low-frequency region corresponding to Mn–O and/or Mn–N vibrations provided additional evidence of successful complex formation. Such spectral behavior has been consistently reported for manganese coordination compounds containing oxygen- and

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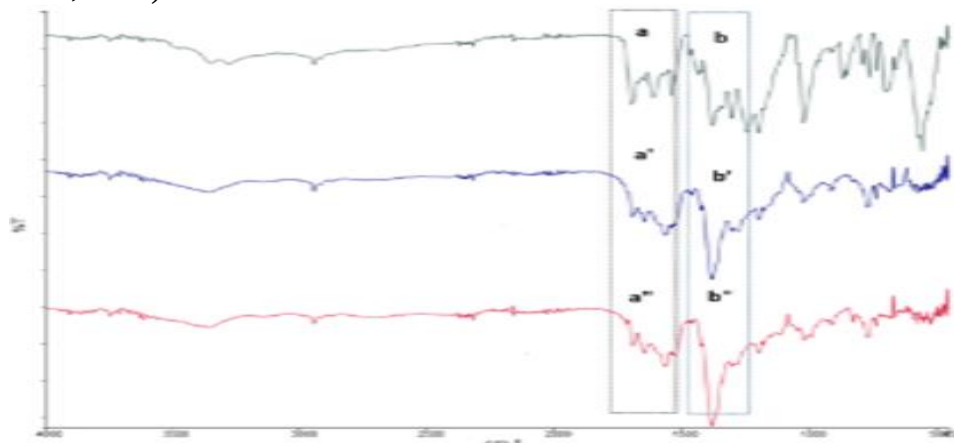
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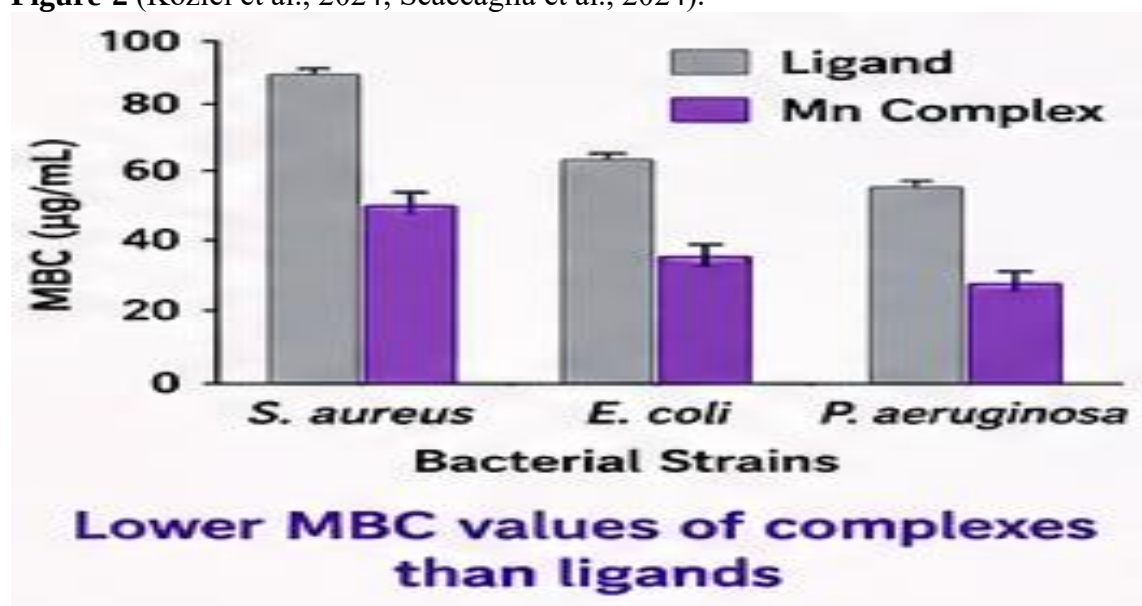
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nitrogen-donor ligands (Juyal et al., 2024). FT-IR and elemental analysis, these findings strongly support the proposed molecular structures of the synthesized manganese complexes. Similar spectroscopic trends have been reported for structurally related manganese complexes with biologically active ligands as shown in **Figure-1** (Dimiza et al., 2024).

**Figure-1**

The enhanced antibacterial activity observed after manganese coordination may be explained by chelation theory, which proposes that coordination reduces the polarity of the metal ion through partial sharing of positive charge with donor atoms while increasing the lipophilic character of the resulting complex. Increased lipophilicity facilitates penetration through bacterial cell membranes and promotes interaction with intracellular biomolecules, thereby enhancing antimicrobial activity as shown in

Figure-2 (Kozieł et al., 2024; Scaccaglia et al., 2024).**Figure-2**

The antibiofilm assay demonstrated that the synthesized manganese complexes

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effectively inhibited bacterial biofilm formation. Biofilms constitute one of the major mechanisms responsible for bacterial persistence and antimicrobial resistance because they protect microorganisms from host immune responses and significantly reduce antibiotic penetration. Consequently, compounds capable of preventing biofilm formation represent promising therapeutic candidates for managing chronic and post-surgical infections (Golden et al., 2024). Recent studies have also shown that manganese-based coordination compounds can effectively inhibit biofilm development while maintaining acceptable biocompatibility as shown in **Figure-3** (Zeng et al., 2024).

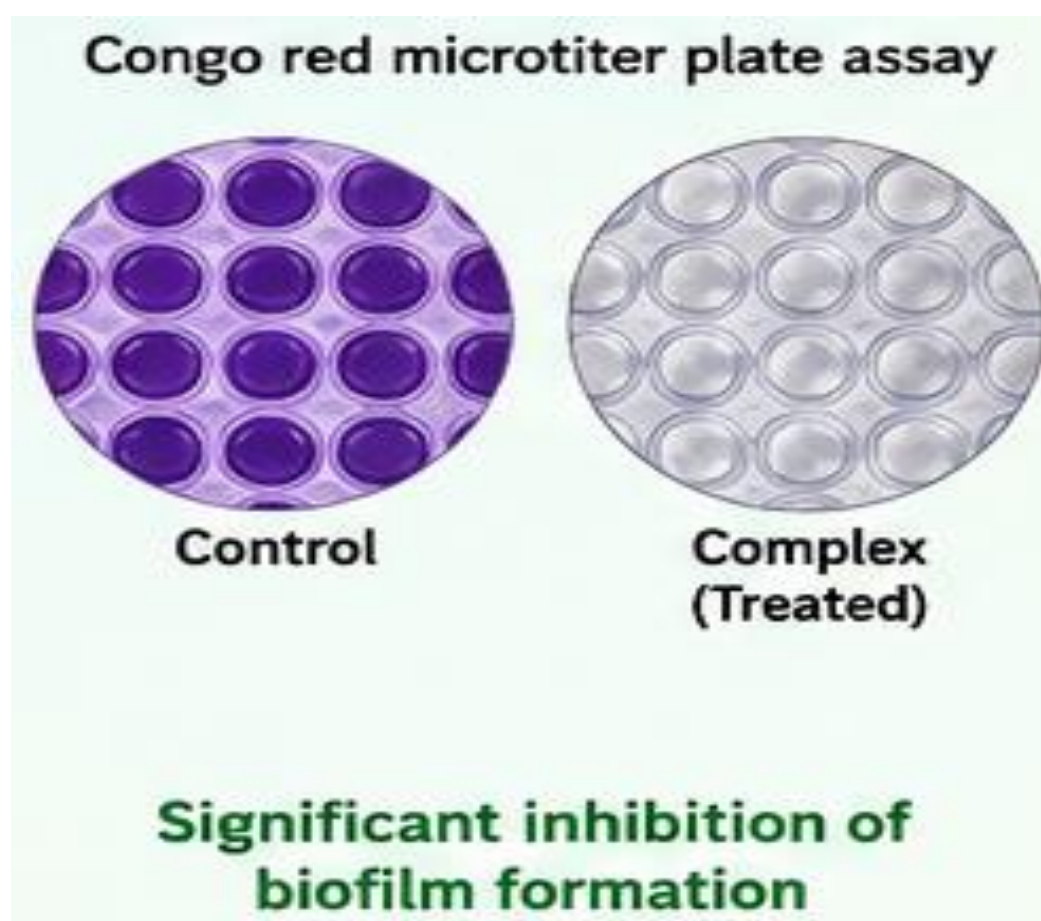


Figure-3

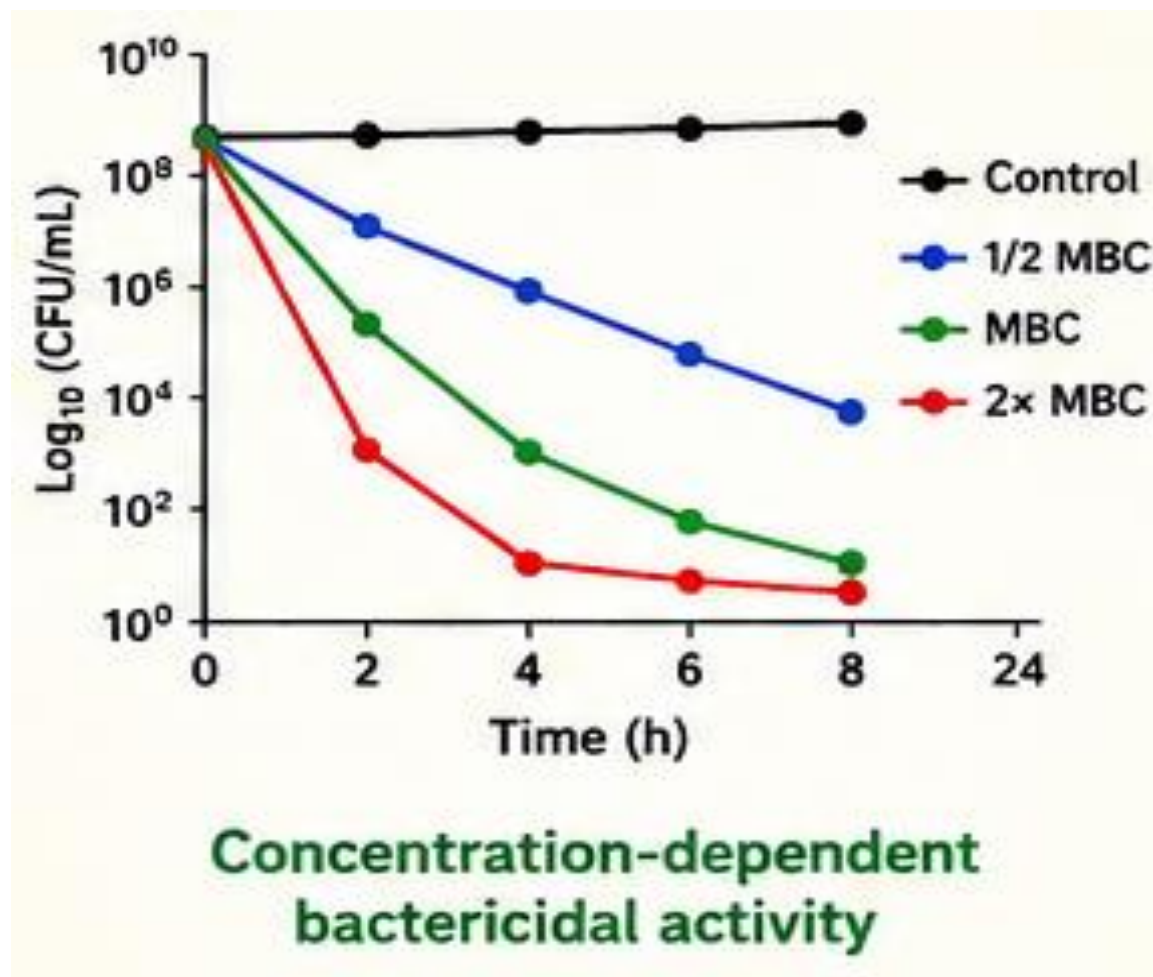
Time-kill experiments further confirmed the bactericidal potential of the synthesized manganese complexes. The progressive reduction in viable bacterial counts over time indicates concentration- and time-dependent antimicrobial activity. Rapid bactericidal action is particularly advantageous for topical antimicrobial agents because it minimizes bacterial colonization and reduces the opportunity for biofilm establishment during wound healing. Comparable bactericidal behavior has been reported for newly synthesized manganese coordination compounds exhibiting potent activity against Gram-positive bacterial pathogens as shown in **Figure-4** (Scaccaglia et al., 2024).

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<http://amresearchreview.com/index.php/Journal/about>**Figure-4**

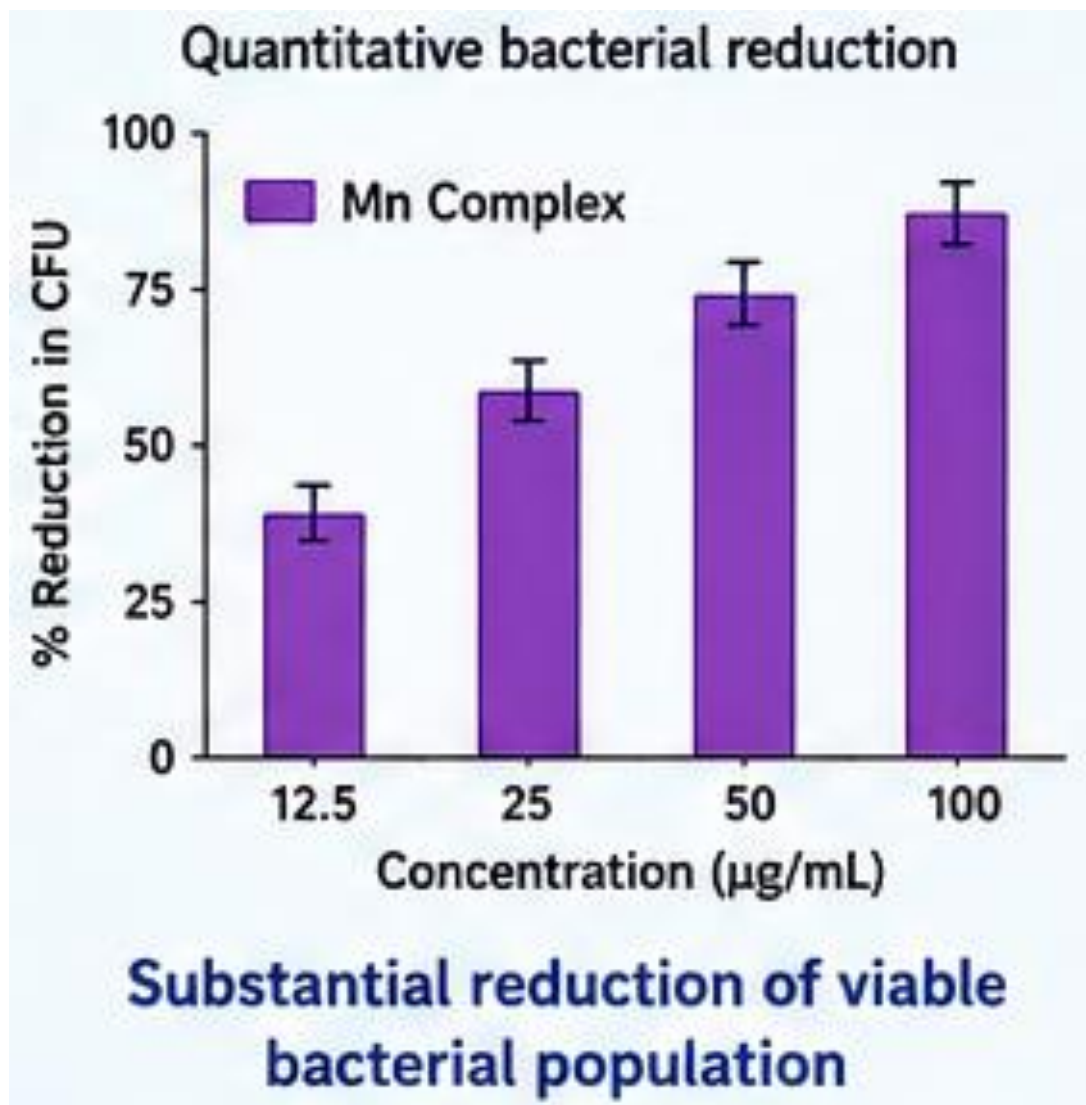
The minimum bactericidal concentration (MBC) values further demonstrated the antimicrobial effectiveness of the synthesized compounds. Lower MBC values generally indicate greater bactericidal potency and stronger interactions with essential bacterial targets. Previous investigations have emphasized that manganese complexes frequently exhibit improved bactericidal properties compared with their corresponding free ligands due to enhanced cellular uptake and multiple intracellular mechanisms of action (Kozieł et al., 2024; Juyal et al., 2024). The shake-flask quantitative bacterial reduction assay also confirmed the remarkable antibacterial performance of the synthesized complexes. Significant reductions in viable bacterial populations under dynamic incubation conditions indicate that the compounds remain biologically active throughout the exposure period. Similar observations have been reported for manganese-based antimicrobial complexes, where sustained bacterial reduction was associated with interference in bacterial respiration, membrane-associated processes, and intracellular metabolic pathways as shown in **Figure-5** (Scaccaglia et al., 2024).

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<http://amresearchreview.com/index.php/Journal/about>**Figure-5**

Several mechanisms may contribute to the superior antibacterial activity of manganese coordination compounds. These include disruption of bacterial membrane integrity, interference with DNA replication, inhibition of essential enzymes, alteration of metal homeostasis, and induction of oxidative stress through reactive oxygen species generation. Because these mechanisms differ from those of many conventional antibiotics, manganese complexes may offer an alternative strategy for combating multidrug-resistant bacterial infections (Golden et al., 2024; Koziel et al., 2024). Recent advances in medicinal inorganic chemistry have further demonstrated that rational ligand design combined with manganese coordination significantly influences antimicrobial potency, biological selectivity, and pharmacological performance. Structural modifications affecting donor atoms, ligand rigidity, and electronic properties may substantially improve biological activity, highlighting the importance of structure–activity relationship studies in the development of next-generation manganese-based therapeutics (Juyal et al., 2024; Scaccaglia et al., 2024). Overall, the

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present study demonstrates that coordination of benzamide-derived ligands with manganese(II) significantly improves their structural and biological characteristics. The synthesized complexes exhibited favorable spectroscopic properties together with promising antibacterial activity, including inhibition of biofilm formation, effective bactericidal action, and substantial quantitative bacterial reduction. These findings support the growing evidence that manganese coordination compounds represent promising candidates for the development of novel antimicrobial agents, particularly for topical management of bacterial infections and prevention of surgical site infections. Future studies should focus on elucidating their molecular mechanisms of action, evaluating cytocompatibility using mammalian cell lines, investigating efficacy against multidrug-resistant clinical isolates, and validating therapeutic performance using **in vivo** wound infection models (Kozieł et al., 2024; Zeng et al., 2024).

CONCLUSION

The present investigation successfully demonstrated the synthesis of novel manganese–benzamide coordination complexes using an environmentally sustainable synthetic methodology. Comprehensive spectroscopic characterization confirmed the successful coordination of the benzamide ligands with manganese(II), providing structural evidence for the formation of stable coordination compounds. Biological evaluation revealed that manganese complexation substantially enhanced the antibacterial properties of the parent ligands, indicating that metal coordination plays a crucial role in improving biological performance. The synthesized complexes exhibited promising antibacterial activity through multiple complementary mechanisms, including effective inhibition of bacterial biofilm formation, concentration-dependent bactericidal activity in time-kill assays, low minimum bactericidal concentrations, and significant quantitative reduction of bacterial populations under shake-flask conditions. These findings suggest that manganese coordination enhances lipophilicity and facilitates stronger interactions with bacterial cellular targets, thereby improving antimicrobial efficacy. The combined spectroscopic and biological results establish a clear relationship between structural modification through metal coordination and enhanced antibacterial activity. Overall, the study highlights manganese–benzamide coordination compounds as promising scaffolds for the development of new metal-based antimicrobial agents, particularly for topical applications and the prevention of surgical site infections. The environmentally friendly synthetic protocol further supports the sustainable development of medicinal coordination compounds while reducing the environmental impact associated with conventional synthetic methods. Future investigations should focus on elucidating the molecular mechanism of antibacterial action, evaluating cytotoxicity toward normal mammalian cells, investigating efficacy against multidrug-resistant clinical isolates, performing detailed structure–activity relationship studies, and validating therapeutic performance using suitable **in vivo** infection and wound-healing models. Such studies will facilitate the translation of these manganese coordination compounds into clinically relevant antimicrobial candidates.

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