Antimicrobial Activities of Functional MXene and Manganese Oxide Transition Metal Oxide Nanoparticles

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**Abstract:** MXenes, a class of 2D transition metal carbides, have gained significant attention due to their unique physicochemical properties. When combined with metal oxides like MnO₂, these hybrid nanomaterials exhibit promising potential in biomedical applications. This study explores the antimicrobial efficacy of functional MXene-MnO₂ composites against a spectrum of microbial pathogens. This study focuses on the synthesis, characterization, and evaluation of the antimicrobial activities of functional MXene-manganese oxide (MnO₂) nanocomposites. Multilayer Ti₃C₂F₂ and Ti₃C₂(OH)₂ MXenes were prepared by selectively etching the Ti₃AlC₂ MAX phase using hydrofluoric acid (HF), with hydrochloric acid (HCl) and dimethyl sulfoxide (DMSO) aiding delamination. MnO₂ nanoparticles were synthesized via an ultrasonication-assisted method, promoting homogenous distribution. The resulting MXene-MnO₂ hybrids were fabricated using a cost-effective and environmentally friendly process. The structural and elemental properties of the composites were analyzed using X-ray Diffraction (XRD), which confirmed the crystalline phases of both MXene and MnO₂. Scanning Electron Microscopy (SEM) revealed layered MXene sheets decorated with evenly dispersed MnO₂ nanoparticles, while Energy Dispersive X-ray Spectroscopy (EDS) confirmed the presence of key elements such as Ti, C, Mn, and O. Antimicrobial testing demonstrated that the MXene-MnO₂ composites exhibited strong antibacterial and antifungal activities, particularly against antibiotic-resistant strains. The enhanced activity is attributed to the synergistic interaction between the high surface area and conductivity of MXene and the oxidative stress-inducing properties of MnO₂. Overall, the MXene-MnO₂ nanocomposite shows great promise as an antimicrobial agent for biomedical and environmental applications.

**Keywords**: Transition Metal Oxides, Nanocomposites, Reactive Oxygen Species, MXene, Manganese Oxide.

# INTRODUCTION

The escalating prevalence of antibiotic-resistant bacterial infections poses a significant threat to global public health, necessitating the exploration of alternative antimicrobial strategies. In this context, nanotechnology offers promising avenues, particularly through the development of nanomaterials with inherent antimicrobial properties. Among these, two-dimensional (2D) transition metal carbides and nitrides, collectively known as MXenes, and manganese oxide (MnO₂) nanoparticles have garnered considerable attention for their potential in combating pathogenic microorganisms[(Iravani & Varma, 2023)](https://paperpile.com/c/qa4r3Z/vwjg)[(Ajay et al., 2023; Chokkattu et al., 2023; Padarthi et al., 2023)](https://paperpile.com/c/qa4r3Z/piC1+WIKe+H1Es)[(Dharman et al., 2023; S. Sindhu et al., 2023; Sreenivasagan et al., 2023)](https://paperpile.com/c/qa4r3Z/sTzZ+Iw1K+gwtu)[(Ramakrishnan et al., 2023; Shenoy & Maiti, 2023; J. S. Sindhu et al., 2023)](https://paperpile.com/c/qa4r3Z/zJWs9+rdcDn+lQaHf)[(Kasabwala et al., 2021; Rajeshkumar & Lakshmi, 2021; Varghese et al., 2023)](https://paperpile.com/c/qa4r3Z/FaoH+5yq0+3bjP)MXenes are a class of 2D materials derived from the selective etching of MAX phases, where 'M' represents an early transition metal, 'A' is an A-group element (typically from groups 13 or 14), and 'X' denotes carbon and/or nitrogen. The general formula for MXenes is Mₙ₊₁XₙTₓ, where 'Tₓ' represents surface terminations such as hydroxyl, oxygen, or fluorine groups. These surface terminations impart hydrophilicity to MXenes, distinguishing them from other 2D materials like graphene[(C P et al., 2024)](https://paperpile.com/c/qa4r3Z/5kYQ). Notably, Ti₃C₂Tₓ is one of the most extensively studied MXenes, exhibiting properties such as high electrical conductivity, large surface area, and mechanical robustness. These attributes make MXenes suitable for various applications, including energy storage, sensors, and, pertinently, antimicrobial agents [(Seidi et al., 2023)](https://paperpile.com/c/qa4r3Z/NSTP)[(Keerthana & Ramesh, 2021; Murugesan, 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/qa4r3Z/mJoAg+ww4z4+3e5By)[(Keerthana & Ramesh, 2021; Murugesan, 2021; Subramanian et al., 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/qa4r3Z/mJoAg+ww4z4+3e5By+3Hfx6)[(*Evaluation Composite Restoration Posterior Teeth Proanthocyanidin Pretreatment Liner Using Fédération Dentaire Internationale Criteria: Split-Mouth Randomized Controlled Trial*, n.d.; Pranati et al., 2021; Sakthi 2021)(*Evaluation Composite Restoration Posterior Teeth Proanthocyanidin Pretreatment Liner Using Fédération Dentaire Internationale Criteria: Split-Mouth Randomized Controlled Trial*, n.d.; Pranati et al., 2021; Sakthi 2021)](https://paperpile.com/c/qa4r3Z/ji7I+kXT4+bQBW))The antimicrobial mechanisms of MXenes are multifaceted. One primary mode involves physical disruption of bacterial cell membranes. The sharp edges of MXene nanosheets can penetrate and compromise the integrity of bacterial membranes, leading to cell lysis and death. Additionally, MXenes can induce oxidative stress by generating reactive oxygen species (ROS), which damage cellular components such as lipids, proteins, and nucleic acids[(Seidi et al., 2023)](https://paperpile.com/c/qa4r3Z/NSTP). Furthermore, the photothermal properties of MXenes enable them to convert light energy into heat upon irradiation, facilitating photothermal therapy (PTT). This localized hyperthermia can effectively inactivate bacteria, including antibiotic-resistant strains.​ Hybridization of MXenes with other materials has been explored to enhance their antimicrobial efficacy. For instance, composites of MXenes with silver nanoparticles have demonstrated synergistic effects, combining the membrane-disrupting capabilities of MXenes with the well-known antibacterial properties of silver. Such composites have shown improved performance in eradicating both Gram-positive and Gram-negative bacteria[(Ye et al., 2024)](https://paperpile.com/c/qa4r3Z/iwgy).​Manganese oxide (MnO₂) nanoparticles represent another class of nanomaterials with notable antimicrobial activity. MnO₂ exists in various polymorphic forms, including α-, β-, γ-, and δ-MnO₂, each exhibiting distinct physicochemical properties[(Huang et al., 2023)](https://paperpile.com/c/qa4r3Z/BdNz). The antimicrobial action of MnO₂ nanoparticles is primarily attributed to their ability to generate ROS, leading to oxidative stress and subsequent bacterial cell damage. Additionally, MnO₂ nanoparticles can disrupt essential metabolic processes within bacterial cells, further contributing to their bactericidal effects. Studies have demonstrated that MnO₂ nanoparticles synthesized via green methods, such as using plant extracts, exhibit effective antibacterial activity against pathogens like Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa [(Suresh et al., 2024)](https://paperpile.com/c/qa4r3Z/WvBj).​The combination of MXenes and MnO₂ nanoparticles into a single nanocomposite is a promising strategy to leverage the unique properties of both materials for enhanced antimicrobial efficacy. Such hybrid nanostructures can integrate the physical membrane-disrupting capabilities and photothermal properties of MXenes with the oxidative stress-inducing characteristics of MnO₂[(Eraky et al., 2024)](https://paperpile.com/c/qa4r3Z/92a2). This synergistic approach may result in a more potent antimicrobial agent capable of effectively targeting a broad spectrum of pathogens, including multidrug-resistant bacteria. Moreover, the tunable surface chemistry and high surface area of these nanocomposites facilitate functionalization with additional antimicrobial agents or targeting ligands, further enhancing their therapeutic potential[(Saod et al., 2022)](https://paperpile.com/c/qa4r3Z/1jDO). The objective of this study is to synthesize and characterize functional MXene and manganese oxide (MnO₂) transition metal oxide nanoparticles, and to evaluate their antimicrobial activities against a broad spectrum of pathogens, including antibiotic-resistant bacteria. By combining the high surface area, conductivity, and membrane-disruptive properties of MXenes with the oxidative stress-inducing potential of MnO₂, the study aims to develop an effective, eco-friendly nanocomposite material with enhanced antimicrobial performance for potential biomedical and environmental applications.

# MATERIALS AND METHODS

## Synthesis of MXene (Ti₃C₂)

2 g of Ti₃AlC₂ powder were gradually added to 40 mL of 40% HF solution under constant stirring in a Teflon vessel. The mixture was stirred at room temperature for 24 hours to etch the aluminum layer away. The resulting mixture was washed several times with Deionized (DI) water by centrifugation at 3500 rpm until the pH of the supernatant became ~6. The sediment was subsequently re-dispersed in DI water and sonicated in an argon atmosphere for 1 hour. Following sonication, the suspension was centrifuged at 3500 rpm for 1 hour to harvest the supernatant carrying delaminated few-layered MXene.

## Synthesis of MnO₂ Nanoparticles

MnO₂ nanoparticles were prepared through a simple redox method. 0.1 M KMnO₄ and 0.05 M Mn(CH₃COO)₂·4H₂O were separately dissolved in DI water. Mn(CH₃COO)₂ solution was dropped slowly into the KMnO₄ solution with constant stirring at room temperature. The reaction mixture was stirred for 4 hours. A dark brown precipitate was observed, indicating MnO₂ formation. The precipitate was centrifuged and washed thoroughly with DI water and ethanol and dried overnight at 60°C.

## Preparation of MXene-MnO₂ Nanocomposite

A stable MXene dispersion (1 mg/mL) was prepared by dispersing delaminated MXene in DI water via ultrasonication. MnO₂ nanoparticles (in stoichiometric ratio or 1:1 weight ratio) were slowly added to the MXene suspension under continuous stirring. The mixture was stirred for 12 hours to facilitate uniform mixing and interaction between the two components. The final product was collected via centrifugation, washed, and dried at 60°C under vacuum.

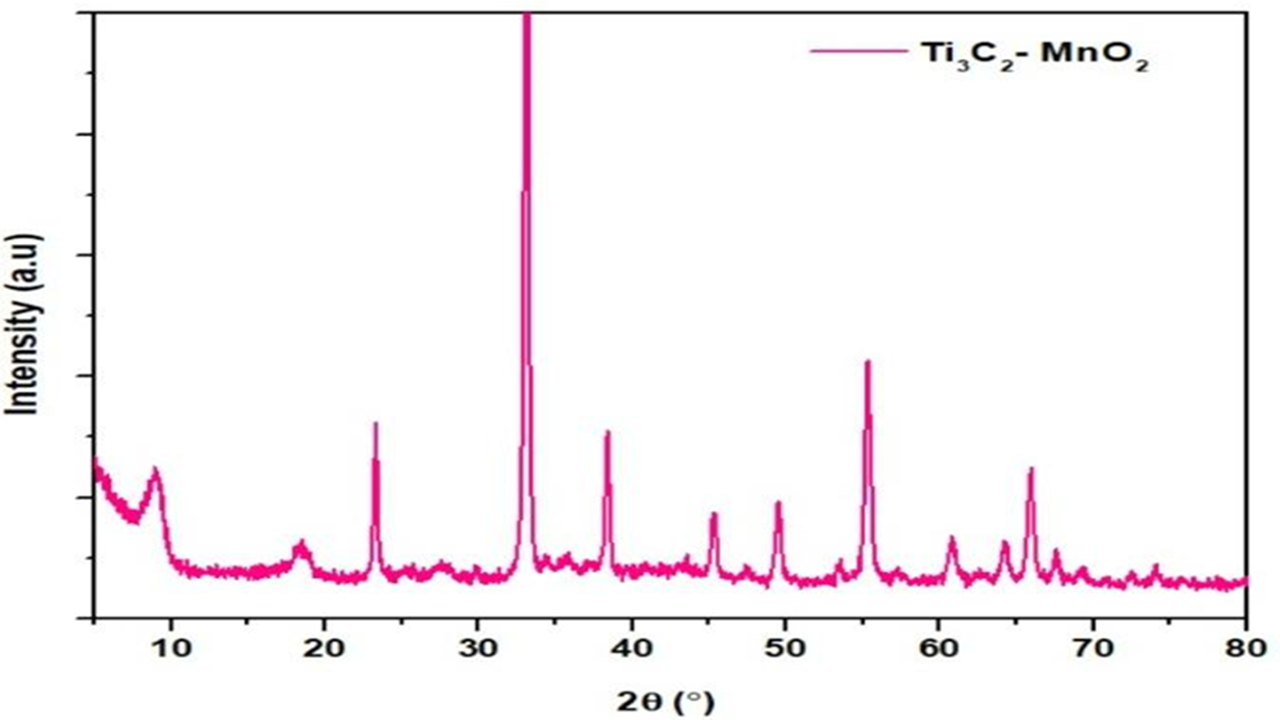
## Antimicrobial Activity

Ti₃C₂-MnO₂ nanoparticles were assessed for their antimicrobial activities through disk diffusion technique. Test solution was prepared by dissolving 50 mg of the nanoparticles in 2.5 mL of ethanol, which was sterilized via a 0.22 mm Millipore filter (Merck, Darmstadt, Germany). The resulting solution was then applied to sterile 8 mm filter paper discs. Subsequently, a base layer of 10 ml Mueller-Hinton agar was poured into sterilized Petri dishes, where nanoparticle-treated discs (10 mg/mL concentration) were placed upon the agar surface. Discs with 20 µg of ketoconazole (for fungi) and 20 µg tetracycline (for bacteria) served as positive controls, whereas blank DMSO was the negative one. The plates were then allowed for 2 hours at 5°C for diffusion of nanoparticles into agar before incubating for 24 h at 35°C for bacterial strains (*Enterococcus faecalis* and *Streptococcus mutans*) and 25°C for fungal strains (*Candida albicans* and *Candida parapsilosis*). Zones of inhibition were measured using a Vernier caliper and analyzed for antimicrobial activity.

# RESULTS AND DISCUSSION

## XRD Analysis

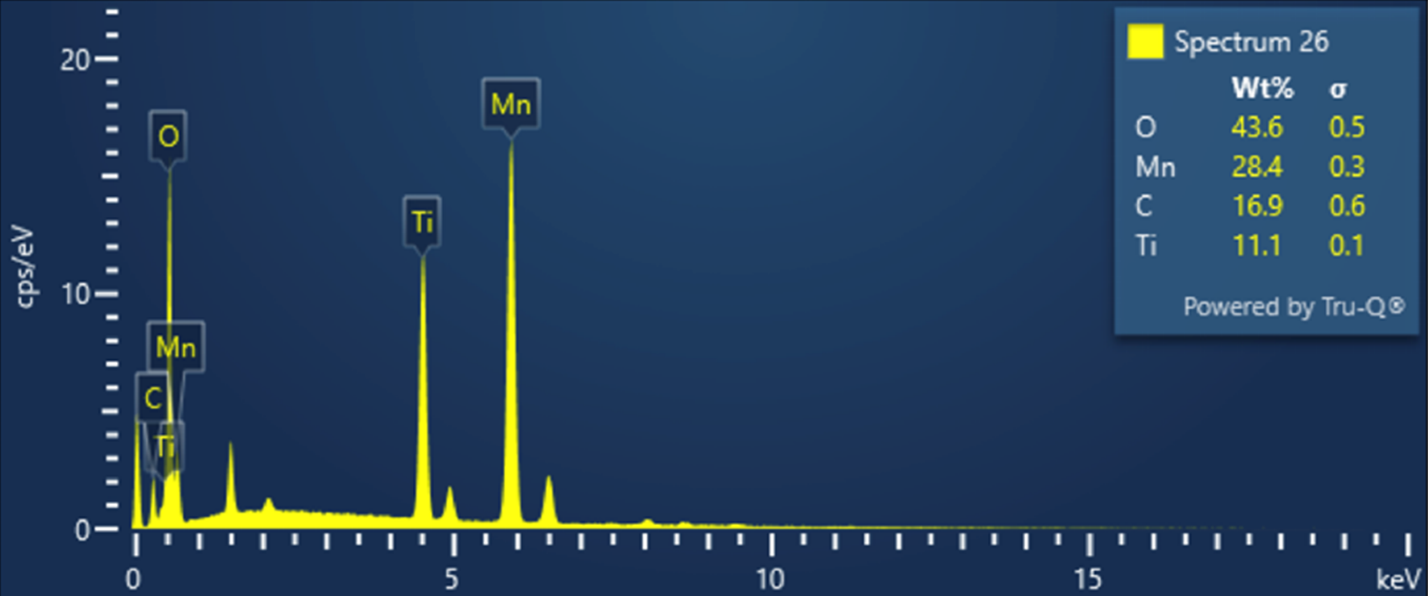
The X-ray diffraction (XRD) pattern of the obtained Ti₃C₂–MnO₂ nanocomposite, as observed in the Figure 1, indicates the crystalline structure and effective decoration of MnO₂ nanoparticles onto the MXene surface. The XRD spectrum shows a few clear peaks from 5° to 80° (2θ), one of which is a very strong peak at about 2θ = 27.4° corresponding to the (002) plane of Ti₃C₂ MXene, which confirms its well-layered structure. The existence of this sharp peak verifies the successful exfoliation and structural retention of MXene after etching and composite formation. The other diffraction peaks appearing in the areas of 2θ = 12.7°, 36.6°, 42.0°, 56.4°, and 66.1° are due to the crystalline forms of manganese dioxide (MnO₂) agreeing with the typical JCPDS card No. 44-0141 relating to the tetragonal crystal structure of MnO₂. These peaks are indicative of the existence of MnO₂ nanoparticles in the composite and attest to the successful fabrication of the Ti₃C₂–MnO₂ nanostructure. Peaks at approximately 36.6° and 56.4° corresponding to the (211) and (510) planes of MnO₂, respectively, reflect an excellent level of crystallinity and purity of phase of the metal oxide. There are no other impurity peaks, which reflect that the synthesis did not lead to any unexpected phases or by-products. In addition, the persistence of MXene characteristic peak at 27.4° as well as the appearance of new peaks arising from MnO₂ signify that the composite formation was done without disturbing the layered MXene structure. The presence of diffraction features of both Ti₃C₂ and MnO₂ together verifies that the nanocomposite maintains the structural integrity of both elements. The XRD spectrum of the successfully synthesized Ti₃C₂–MnO₂ nanocomposite verifies that MnO₂ nanoparticles have successfully been integrated into the MXene surface without destruction of the crystalline structure of the two entities. The dominating peak at about 2θ = 27.4° is due to the (002) plane of Ti₃C₂ MXene, which evidences its perfectly ordered layered arrangement and partial restacking upon exfoliation[(Zhang et al., 2020)](https://paperpile.com/c/qa4r3Z/wjtK). The appearance of characteristic MnO₂ peaks at 2θ values of 12.7°, 36.6°, 42.0°, 56.4°, and 66.1° correlates well with the standard JCPDS card No. 44-0141, indicating the formation of crystalline MnO₂ with a tetragonal phase. Such diffraction peaks, especially for the (211) and (510) planes, indicate high crystallinity as well as phase purity of MnO₂ in the composite[(Zhu et al., 2022)](https://paperpile.com/c/qa4r3Z/xGlt). The presence of both Ti₃C₂ and MnO₂ phases in the diffractogram together, without showing any secondary impurity peaks, reflects a successful composite formation without degenerating the core structures of either material. Additionally, the lack of peaks associated with Ti₃AlC₂ MAX phase ensures thorough etching of aluminum layers during MXene synthesis. These results confirm that the structural stability and synergistic nature of both MXene and MnO₂ were preserved in the composite, yielding a potential platform for antimicrobial applications owing to improved surface reactivity and functional properties[(Luo et al., 2013)](https://paperpile.com/c/qa4r3Z/7QIN).



**Figure 1:** XRD Analysis of Ti₃C₂–MnO₂ nanocomposite

## EDS Analysis

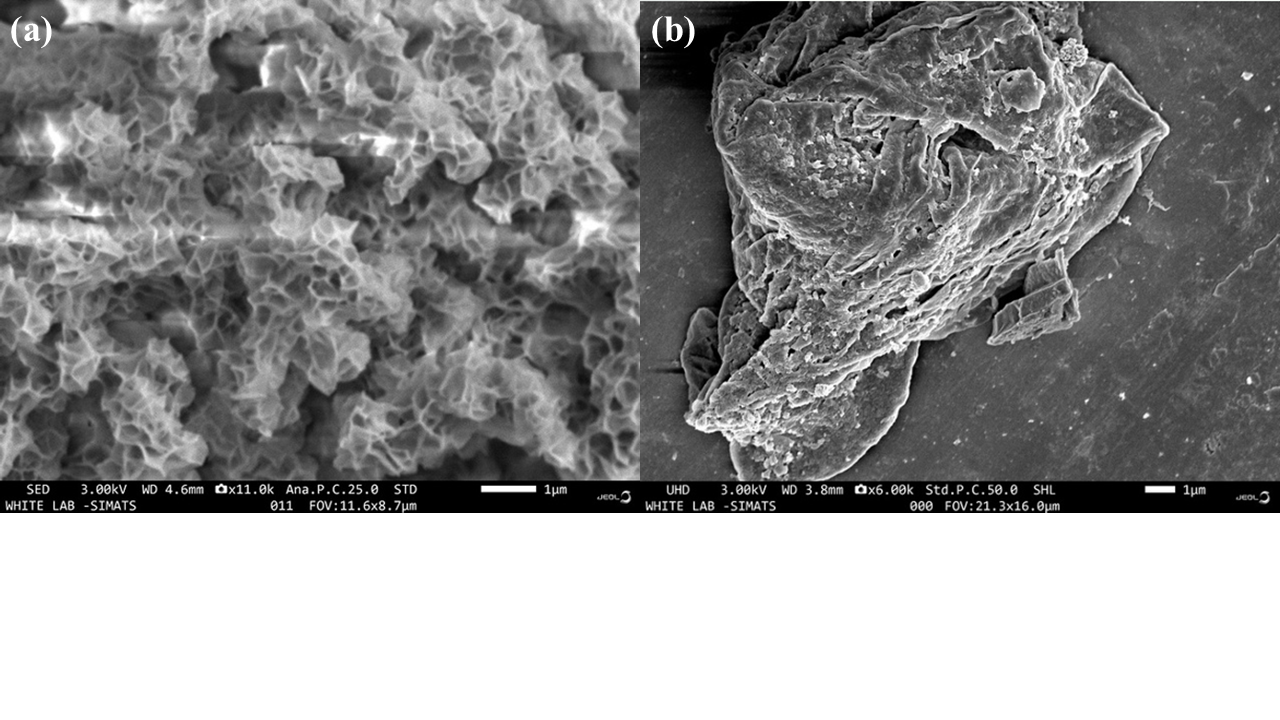
The energy-dispersive X-ray spectroscopy (EDS) spectrum of the MXene-MnO₂ nanocomposite, as indicated in the given spectrum figure 2, verifies the successful decoration of manganese oxide on the MXene (Ti₃C₂) surface. The elemental constituents detected in the sample are oxygen (O), manganese (Mn), carbon (C), and titanium (Ti) with respective weight percent compositions of 43.6%, 28.4%, 16.9%, and 11.1%. The large peak of oxygen indicates the existence of oxygen-rich manganese oxide, which is in agreement with the MnO₂ stoichiometry expected and advocates for its formation on the MXene substrate. The high content of Mn also confirms the successful decoration or loading of MnO₂ nanoparticles on the Ti₃C₂ nanosheets. The peak of carbon aligns with the layered carbon matrix of the MXene sheets, which is derived from the selective etching of the MAX phase (Ti₃AlC₂) to take out aluminum. The high content of titanium is a pointer to the retained Ti₃C₂ structure in the composite. Interestingly, the absence of aluminum in the spectrum is a pointer to the total etching of the A-layer element during MXene synthesis. The observation of prominent Mn and Ti peaks illustrates the successful hybridization of MnO₂ onto the MXene surface. The significant presence of oxygen and manganese confirms the successful incorporation of MnO₂, which is essential for enhancing the electrochemical and antimicrobial properties of the composite[(Chavan et al., 2023)](https://paperpile.com/c/qa4r3Z/hIst). The carbon and titanium signals originate from the Ti₃C₂ MXene matrix, indicating the structural integrity of the MXene even after surface modification with MnO₂. The absence of aluminum in the spectrum confirms complete etching of the Al layer from the Ti₃AlC₂ MAX phase during MXene synthesis . The high oxygen content also supports the formation of surface terminations such as –O and –OH, which are characteristic of delaminated MXenes and may enhance interfacial bonding with MnO₂ nanoparticles. This homogeneous distribution of elements and the absence of foreign impurities demonstrate the purity and uniformity of the nanocomposite. The EDS findings complement the XRD results, confirming the successful synthesis of the MXene-MnO₂ hybrid structure with potential applications in antimicrobial and energy-related fields[(Abdullah et al., 2023)](https://paperpile.com/c/qa4r3Z/PpKB).



**Figure 2:** EDS Analysis of Ti₃C₂–MnO₂ nanocomposite

## SEM Analysis

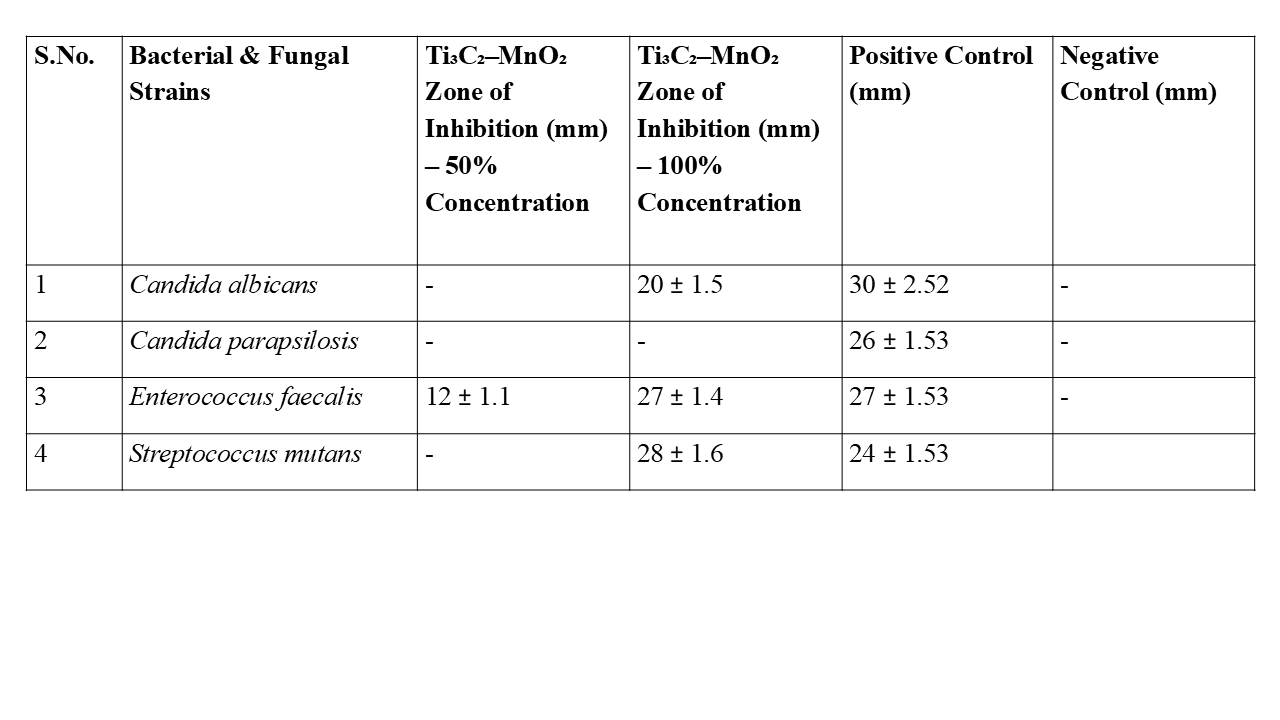
The scanning electron microscopy (SEM) images of the MXene-MnO₂ nanocomposites provide valuable insights into the morphological features and surface architecture of the synthesized material. Figure 3 (a), recorded at a magnification of 11,000x, reveals a highly porous, interconnected network structure. This porous morphology is indicative of the successful anchoring of MnO₂ nanoparticles onto the surface and interlayers of the exfoliated MXene sheets. The open, sponge-like structure facilitates ion transport and provides a large surface area, which is highly beneficial for applications such as supercapacitors, sensors, and antimicrobial agents. The folding and crumpling of nanosheets observed in this region are typical of delaminated MXenes and suggest good dispersion of MnO₂ without aggregation, enabling enhanced surface reactivity. In contrast, Figure 3 (b) recorded at 6,000x magnification, displays the bulk morphology of a typical MXene-MnO₂ composite flake. The layered arrangement visible here reflects the inherent two-dimensional nature of MXene, where MnO₂ particles appear to be embedded or coated on the surface(Saadh et al., 2024). The layered sheets exhibit a rough texture and slight curling at the edges, further confirming the exfoliated nature of the MXene layers and effective MnO₂ decoration(Almatrafi et al., 2024). This layered-sheet configuration is crucial in preserving the high electrical conductivity and mechanical strength of MXene while incorporating the pseudocapacitive and antimicrobial properties of MnO₂. The SEM analysis of MXene-MnO₂ nanocomposites exhibit a crumpled, layered morphology characteristic of exfoliated MXene sheets, which are interspersed with MnO₂ particles. The porous and wrinkled texture indicates successful intercalation and surface deposition of MnO₂, which helps in preventing the restacking of MXene layers, thereby preserving a high surface area. Such morphology is essential for applications requiring efficient ion transport and electron conductivity, such as supercapacitors and sensors[(Ramkumar et al., 2024)](https://paperpile.com/c/qa4r3Z/BncN). The uniform dispersion of MnO₂ nanoparticles across the MXene matrix suggests strong interfacial interactions, which can enhance structural stability and electrochemical performance. The layered and slightly curled edges visible in the SEM images also affirm the two-dimensional nature of MXene and its compatibility with MnO₂. This hybrid nanostructure facilitates synergistic effects by combining the electrical conductivity of MXene with the pseudocapacitive behavior and oxidative functionality of MnO₂. Additionally, the porous architecture formed through this integration improves electrolyte accessibility, which is beneficial for charge storage and catalytic applications[(Xi et al., 2023)](https://paperpile.com/c/qa4r3Z/FbM2).



**Figure 3:** SEM Analysis of Ti₃C₂–MnO₂ nanocomposite

## Antimicrobial Activity

The antimicrobial activity of Ti₃C₂–MnO₂ nanocomposites was evaluated against various oral pathogens and fungal species, including *Streptococcus mutans*, *Enterococcus faecalis*, *Candida albicans*, and *Candida parapsilosis*, using the zone of inhibition assay at two different concentrations (50% and 100%) in Figure 4 (Table 1). According to the data, *Streptococcus mutans*, a key contributor to dental caries, showed no inhibition at 50% concentration of the nanocomposite, but exhibited a significant zone of inhibition of 28 ± 1.6 mm at 100% concentration. This antimicrobial performance surpassed the positive control (24 ± 1.53 mm), indicating that the nanocomposite is highly effective against this bacterium when used at full concentration. Similarly, *Enterococcus faecalis*, a resilient pathogen commonly associated with failed root canal treatments, showed a moderate inhibition zone of 12 ± 1.1 mm at 50% concentration and a markedly enhanced zone of 27 ± 1.4 mm at 100% concentration, which is comparable to the positive control (27 ± 1.53 mm). This suggests a concentration-dependent antibacterial effect of the nanocomposite, where higher doses are required for optimal efficacy. In contrast, fungal strains exhibited varied responses. Candida albicans, a common oral opportunistic fungus, showed no inhibition at 50% concentration but a notable zone of 20 ± 1.5 mm at 100% concentration, although this was still lower than the inhibition observed with the positive control (30 ± 2.52 mm). This indicates moderate antifungal activity at higher concentrations. Candida parapsilosis showed no inhibition at either concentration, demonstrating that the nanocomposite was ineffective against this particular strain under the tested conditions. Overall, Ti₃C₂–MnO₂ nanocomposites demonstrated strong antimicrobial effects against the tested bacterial pathogens, particularly at higher concentrations, with limited but selective antifungal activity. These findings suggest potential for their use in dental or biomedical applications targeting resistant oral bacteria[(Tamhane et al., 2024)](https://paperpile.com/c/qa4r3Z/rVb0). Studies have shown that Ti₃C₂ MXenes can exhibit strong antibacterial effects against both Gram-positive and Gram-negative bacteria, including Escherichia coli and Bacillus subtilis. Moreover, Ti₃C₂ has demonstrated the capacity to generate reactive oxygen species (ROS), further enhancing its bactericidal effect[(Rasool et al., 2016)](https://paperpile.com/c/qa4r3Z/k3mD).MnO₂ nanoparticles also possess intrinsic antimicrobial activity due to their redox properties and ability to catalyze ROS generation. These particles can interact with bacterial cell walls, disrupt membrane potential, and interfere with intracellular components. For instance, MnO₂ nanoparticles have shown effective antimicrobial behavior against Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans when incorporated into nanocomposites. The synergistic integration of Ti₃C₂ with MnO₂ potentially enhances antimicrobial efficiency through combined physical disruption and oxidative mechanisms. Such composites have been reported to produce notable inhibition zones against oral pathogens like Streptococcus mutans and Enterococcus faecalis, as well as fungal species including Candida parapsilosis and Candida albicans[(Velho-Pereira & Parmekar, 2024)](https://paperpile.com/c/qa4r3Z/o7kJ).



**Figure 4** (Table 1): Zone of Inhibition by Ti₃C₂-MnO₂ on Different Bacterial and Fungal Strains

The positive control confirms that the assay can detect antimicrobial activity, while the negative control confirms that the observed inhibition in other samples is due to the test compounds and not due to any artefact or contamination. Positive control for *C. albicans* and *C. parapsilosis* is ketoconazole and *E. faecalis* and *S. mutans* is tetracycline. Negative control for *C. albicans, C. parapsilosis, E. faecalis* and *S. mutans* is DMSO (dimethyl sulfoxide).

# CONCLUSION

This study demonstrated the antimicrobial potential of functionalized MXene and manganese oxide (MnO) transition metal oxide nanoparticles, characterized using X-ray diffraction (XRD), energy-dispersive X-ray spectroscopy (EDS), and scanning electron microscopy (SEM). XRD confirmed the crystalline structure and purity, while SEM revealed nanoscale morphology with well-dispersed particles, and EDS validated elemental composition. These structural features play a crucial role in the antimicrobial mechanisms of the nanoparticles. Antimicrobial activity tests using bacterial and fungal strains showed significant inhibition, indicating strong bactericidal and fungicidal properties. MXene nanoparticles exhibited superior activity, likely due to their high surface area, sharp edges, and conductivity, enhancing interaction with microbial membranes and promoting oxidative stress. MnO nanoparticles also demonstrated effective antimicrobial behavior. The combined results highlight the potential of these nanomaterials as alternatives to traditional antimicrobial agents, particularly in combating antibiotic-resistant pathogens. This work lays a foundation for further research into their application in medical and environmental antimicrobial solutions.

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