Hydrothermal Synthesis of MXene–BiWO₄ Nanocomposites for Enhanced Antibacterial and Cytotoxic Activity

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**ABSTRACT :**The development of novel nanocomposites with superior biological activity has garnered increasing attention for biomedical applications. Among these, MXenes two-dimensional transition metal carbides and nitrides have emerged as promising candidates due to their unique electrical, mechanical, and chemical properties. Coupling MXenes with bismuth tungstate (BiWO₄), a visible-light-active semiconductor, can lead to synergistic enhancements in antimicrobial and cytotoxic efficacy. In this study, Ti₃C₂-BiWO₄ nanocomposites were synthesized via a controlled hydrothermal method, ensuring uniform distribution and intimate interaction between the two components. The synthesis involved in situ growth of BiWO₄ nanoparticles on delaminated MXene sheets, yielding a stable hybrid structure. Comprehensive characterization of the nanocomposites was conducted using X-ray diffraction (XRD), energy-dispersive X-ray spectroscopy (EDS), and scanning electron microscopy (SEM), confirming successful integration and revealing a well-dispersed morphology with enhanced crystallinity. Antibacterial tests demonstrated significant inhibition against both Gram-positive and Gram-negative bacteria, surpassing the activity of individual components. Cytotoxic studies performed on selected cancer cell lines revealed pronounced cell viability reduction, indicating the potential of the composite for anticancer applications. The combined properties of high surface area, active sites, and synergistic mechanisms contribute to the enhanced bioactivity of the Ti₃C₂-BiWO₄ system. This work highlights the efficacy of hydrothermally synthesized Ti₃C₂-BiWO₄ nanocomposites as multifunctional agents with potential utility in antimicrobial and anticancer therapies. Future studies may explore their mechanism of action in greater detail and assess biocompatibility and performance in in vivo systems to advance their translational potential in biomedical fields.

**Keywords**: MXene–BiWO₄ nanocomposites, Hydrothermal synthesis, Antibacterial activity, Synergistic bioactivity, Reactive Oxygen Species.

# INTRODUCTION

The development of multidrug-resistant pathogens and the growing incidence of cancer have created a need for the creation of novel materials with enhanced antimicrobial and cytotoxic activities. In this regard, nanotechnology has facilitated the design of multifunctional nanocomposites that possess outstanding physicochemical properties and biological activities[(Khan et al., 2019)](https://paperpile.com/c/3WztoA/C1w6). Among such nanomaterials, MXenes, a group of two-dimensional (2D) transition metal carbides, nitrides, or carbonitrides have drawn considerable interest owing to their peculiar layered structure, high surface area, electrical conductivity, and chemical functionality[(Naguib et al., 2011)](https://paperpile.com/c/3WztoA/El1K). MXenes, first found in 2011, are generally produced through selective etching of A-elements (generally group IIIA or IVA elements like Al or Si) from the MAX phases, which are ternary carbides or nitrides with the general formula Mn+1AXn, where M is an early transition metal, A is an A-group element, and X is carbon or nitrogen. The resulting MXenes, e.g., Ti₃C₂Tx (where Tx refers to surface terminations such as -OH, -F, or =O), consist of layered nanosheets with excellent hydrophilicity and tunable surface chemistry and are appropriate for a variety of applications, including biomedical applications[(Dhamodharan et al., 2024)](https://paperpile.com/c/3WztoA/nSx3).BiWO₄ (bismuth tungstate) is another material with great potential that has garnered tremendous research attention based on its photocatalytic and antibacterial characteristics. BiWO₄ typically crystallizes in a monoclinic scheelite structure, where layers of corner-sharing BiO₈ and WO₆ polyhedra are the characteristic features [(Ramakrishnan et al., 2023; Shenoy & Maiti, 2023; J. S. Sindhu et al., 2023)](https://paperpile.com/c/3WztoA/1H3aV+tD1gf+dryHw). Its layered architecture is responsible for its good charge separation efficiency, which is highly desirable in applications such as photocatalysis and reactive oxygen species (ROS) generation[(Zhao et al., 2005)](https://paperpile.com/c/3WztoA/Bo6k). The large band gap of BiWO₄ (circa 2.7–2.8 eV) and visible light absorbing capability further increase its applicability in environmental and biological applications. But the intrinsic shortcomings of BiWO₄, like its low surface area and recombination of charges, have led to attempts at combining it with other nanomaterials in order to improve its efficiency [(Dharman et al., 2023; S. Sindhu et al., 2023; Sreenivasagan et al., 2023)](https://paperpile.com/c/3WztoA/c5XYd+5B4CN+8Vpn9).The combination of Ti₃C₂-BiWO₄ into a nanocomposite through hydrothermal synthesis presents an innovative method of addressing the specific weaknesses of each component while enhancing their combined characteristics synergistically. Hydrothermal processing is a practical means of crystalline nanocomposite fabrication with controlled temperature and pressure conditions that commonly yields crystalline materials of uniform morphology, high purity, and enhanced structural coherence[(Qu et al., 2020)](https://paperpile.com/c/3WztoA/T9zo). By introducing MXenes into a BiWO₄ matrix, the composite material takes advantage of the conductive channels offered by the MXene layers to promote charge transfer and inhibit electron–hole recombination. Such improvement is especially useful in biomedical applications where ROS generation is important for antimicrobial and cytotoxic activities [(Ajay et al., 2023; Chokkattu et al., 2023; Padarthi et al., 2023)](https://paperpile.com/c/3WztoA/3jdv0+bac17+gzQvP).Antimicrobial activity refers to the ability of a substance to inhibit the growth or destroy pathogenic microorganisms, including bacteria, fungi, and viruses. Nanomaterials have emerged as potent antimicrobial agents due to their ability to interact with microbial membranes, generate ROS, and release metal ions that disrupt cellular processes[(Alhamada et al., 2022)](https://paperpile.com/c/3WztoA/HEh0). For Ti₃C₂-BiWO₄ nanocomposites, the antimicrobial activity is thought to be greatly enhanced due to the production of ROS upon light irradiation, which destroys microbial cell walls and intracellular structures. Additionally, the high surface area and sharp edges of MXenes enable them to physically engage with and penetrate bacterial membranes, causing membrane disruption and leakage of cellular materials. The interaction between these mechanical and chemical effects is what creates an effective antimicrobial effect, most especially against both Gram-positive and Gram-negative bacteria[(Ye et al., 2024)](https://paperpile.com/c/3WztoA/ANOF).Cytotoxicity, on the contrary, entails the capability of an agent to destroy or inhibit growth of cancer or abnormal cells. Ti₃C₂-BiWO₄ nanocomposites have shown great cytotoxicity by causing oxidative stress in cancer cells through the generation of ROS, resulting in mitochondrial damage, DNA fragmentation, and eventually apoptosis (programmed cell death). MXene biocompatibility and surface functionalization also enable targeted delivery and controlled release of therapeutic agents, thus minimizing systemic toxicity[(Liaqat et al., 2024)](https://paperpile.com/c/3WztoA/ItEg). Moreover, the photo-responsiveness of BiWO₄ facilitates photodynamic therapy applications where local light stimulation amplifies ROS-mediated cytotoxicity in cancer tissues [(Kasabwala et al., 2021; Rajeshkumar & Lakshmi, 2021; Varghese et al., 2023)](https://paperpile.com/c/3WztoA/Qr38F+9ZlPZ+KtA7H). This multifunctionality makes Ti₃C₂-BiWO₄ nanocomposites most appropriate for innovative cancer treatment techniques. This study aims to synthesize Ti₃C₂-BiWO₄ nanocomposites via hydrothermal methods and evaluate their structural, elemental, and morphological properties using XRD, EDS, and SEM, while investigating their enhanced antimicrobial and cytotoxic activity to explore their potential as next-generation agents in biomedical applications.

# MATERIALS AND METHODS

## Synthesis of Ti₃C₂–BiWO₄ nanocomposites

The synthesis of Ti₃C₂–BiWO₄ nanocomposites involves a two-step process comprising the preparation of Ti₃C₂ MXene followed by the in-situ growth of BiWO₄ via a hydrothermal method. Initially, 2 g of Ti₃AlC₂ powder was gradually added to 40 mL of 40% hydrofluoric acid (HF) under continuous stirring in a Teflon vessel and allowed to react at room temperature for 24 hours to selectively etch the aluminum layers, resulting in multilayered Ti₃C₂ MXene. The resultant mixture was centrifuged at 3500 rpm and washed several times with deionized (DI) water until the pH of the supernatant reached approximately 6. The sediment was re-dispersed in DI water and subjected to sonication for 1 hour in an argon atmosphere to facilitate delamination. After sonication, the suspension was centrifuged again at 3500 rpm for 1 hour to collect the supernatant containing few-layered Ti₃C₂, which was then vacuum-dried to obtain the exfoliated Ti₃C₂ powder. For the synthesis of BiWO₄, 1.96g of bismuth nitrate pentahydrate (Bi(NO₃)₃·5H₂O) and 0.67g of sodium tungstate dihydrate (Na₂WO₄·2H₂O) were separately dissolved in deionized water under magnetic stirring, then combined to form a homogeneous precursor solution. The pH of the mixture was adjusted to around 7 using a dilute sodium hydroxide solution. A pre-measured amount of Ti₃C₂ powder was dispersed ultrasonically in DI water to obtain a stable suspension, which was added dropwise into the BiWO₄ precursor solution under vigorous stirring to ensure uniform distribution and interaction between the MXene sheets and BiWO₄ components. The resulting suspension was transferred into a Teflon-lined stainless-steel autoclave and subjected to hydrothermal treatment at 160–180°C for 12–24 hours to facilitate the in-situ growth of BiWO₄ nanoparticles onto the Ti₃C₂ nanosheets. After the reaction, the autoclave was cooled naturally to room temperature, and the final product was collected by centrifugation, thoroughly washed with ethanol and DI water to remove impurities, and dried in a vacuum oven at 60°C. The obtained Ti₃C₂–BiWO₄ nanocomposite appeared as a dark powder, confirming successful hybridization.

## Antibacterial Activity

The antibacterial activity of Ti₃C₂–BiWO₄ nanocomposites was evaluated using the disk diffusion technique. A 50 mg sample of the nanocomposites was dissolved in 2.5 mL of ethanol, sterilized through a 0.22 mm Millipore filter (Merck), and applied onto sterile 8 mm filter paper discs to achieve the desired concentration. Mueller-Hinton agar (10 mL) was poured into sterilized Petri dishes to form the base layer. The prepared discs, containing 10 mg/mL of Ti₃C₂–BiWO₄, were then placed on the agar surface. Discs with 20 µg of tetracycline served as the positive control. The plates were stored at 5°C for two hours to facilitate diffusion of the nanocomposites, followed by incubation at 35°C for 24 hours. Zones of inhibition were measured with a Vernier caliper to determine antibacterial effectiveness.

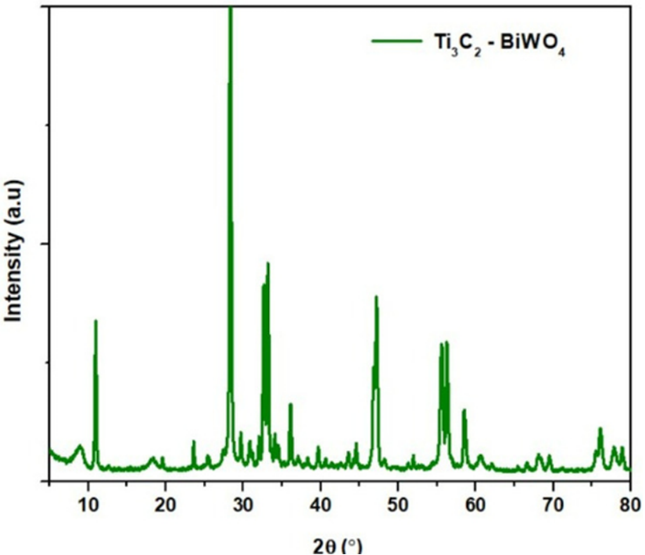
## Cytotoxic Activity

The cytotoxic activity of Ti₃C₂–BiWO₄ nanocomposites was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) assay, a standard colorimetric technique used to measure cell viability and proliferation. In this assay, human colorectal cancer (HCT116) cells were seeded into 96-well plates and allowed to adhere overnight in a humidified CO₂ incubator at 37°C. Following adherence, the cells were treated with varying concentrations of Ti₃C₂–BiWO₄ nanosheets, ranging from very low to high doses, to evaluate dose-dependent cytotoxic effects. Untreated wells served as the negative control, while wells containing only culture media without cells were used as vehicle controls. The cells were incubated with the nanocomposites for 48 hours, providing sufficient time for the material to exert its cytotoxic or antiproliferative effects. After the exposure period, 20 µL of MTT solution (typically 5 mg/mL in PBS) was added to each well, and the plates were further incubated for 2–4 hours at 37°C. During this period, viable and metabolically active cells reduced the yellow MTT reagent to insoluble purple formazan crystals via mitochondrial dehydrogenase activity. Following incubation, the supernatant containing the MTT reagent and culture media was carefully removed, and 100 µL of dimethyl sulfoxide (DMSO) was added to each well to solubilize the formazan crystals.The resulting purple-colored solution was quantified by measuring the absorbance at 570 nm using a microplate reader. The intensity of the color correlates with the number of viable cells. Cell viability was calculated as a percentage relative to the untreated control group. A clear dose-dependent decrease in absorbance values was observed, indicating that Ti₃C₂–BiWO₄ nanocomposites exhibit significant cytotoxicity toward HCT116 cells.

# RESULTS AND DISCUSSION

## XRD Analysis

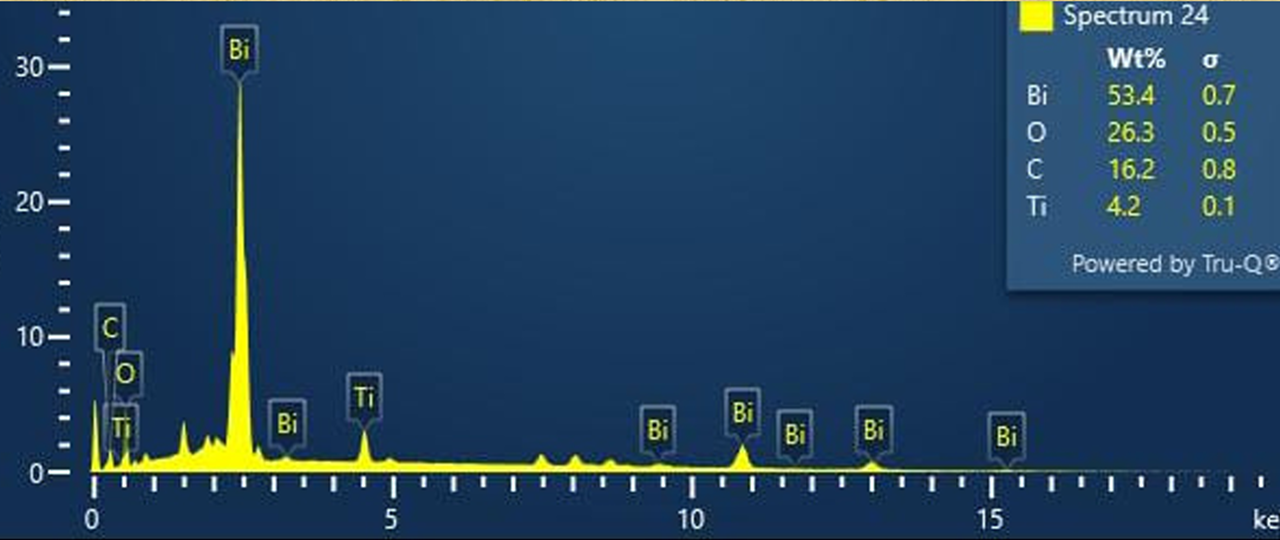
The X-ray diffraction (XRD) pattern of the Ti₃C₂–BiWO₄ nanocomposite, as shown in the Figure 1, provides crucial insight into the crystallographic structure and successful formation of the hybrid material. The diffraction peaks observed in the 2θ range of 5° to 80° clearly reveal the characteristic crystalline phases corresponding to both Ti₃C₂ and BiWO₄. The sharp and intense peaks indicate a well-crystallized composite structure. The dominant peaks located near 28.3°, 32.8°, 47.1°, 55.8°, and 58.5° are attributed to the orthorhombic phase of BiWO₄, which is consistent with the standard Joint Committee on Powder Diffraction Standards (JCPDS) card no. 39-0256. These peaks correspond to the (121), (040), (200), (161), and (321) planes, respectively. The presence of these reflections confirms the successful formation of the BiWO₄ phase in the nanocomposite. BiWO₄ exhibits high crystallinity, and its distinctive orthorhombic structure contributes to enhanced photocatalytic and antimicrobial activity due to efficient electron-hole separation across its lattice. In addition, peaks corresponding to the Ti₃C₂ MXene phase are also visible, particularly the low-angle reflection near 9°, which is characteristic of the (002) plane of Ti₃C₂, indicating successful etching and exfoliation of the MAX phase (Ti₃AlC₂) during the synthesis process. This shift to lower angles is commonly associated with the increase in interlayer spacing due to surface termination with functional groups such as –OH, –O, and –F during MXene synthesis. The absence of any impurity peaks suggests the high purity of the synthesized nanocomposite. The integration of BiWO₄ into the layered structure of Ti₃C₂ likely leads to a synergistic interaction between the two phases, enhancing structural stability and facilitating charge transport. XRD analysis of Ti₃C₂–BiWO₄ nanocomposites confirms the successful integration of BiWO₄ nanoparticles onto the Ti₃C₂ MXene sheets, forming a crystalline hybrid structure. The prominent peaks observed at 2θ values around 28.3°, 32.8°, 47.1°, and 55.8° correspond to the orthorhombic phase of BiWO₄, which aligns with the JCPDS card no. 39-0256, indicating its high crystallinity and phase purity. Additionally, a low-angle peak near 9° is characteristic of the (002) plane of Ti₃C₂, confirming the formation of exfoliated MXene with increased interlayer spacing, a result of surface functionalization during etching[(Naguib et al., 2011)](https://paperpile.com/c/3WztoA/El1K). The absence of secondary phases suggests effective hydrothermal synthesis without impurity formation. The presence of both components in the XRD profile validates the formation of a stable nanocomposite, where Ti₃C₂ acts as a conductive support, and BiWO₄ provides visible-light-responsive photocatalytic properties. This hybrid structure facilitates improved electron-hole separation and charge transfer, which is beneficial for antimicrobial and cytotoxic activities. The structural confirmation through XRD supports the functional role of the Ti₃C₂–BiWO₄ composite in biomedical applications by ensuring its crystalline integrity and synergistic properties[(Mansour et al., 2020)](https://paperpile.com/c/3WztoA/SriV).



**Figure 1:** XRD Analysis of Ti₃C₂–BiWO₄ nanocomposites

## EDS Analysis

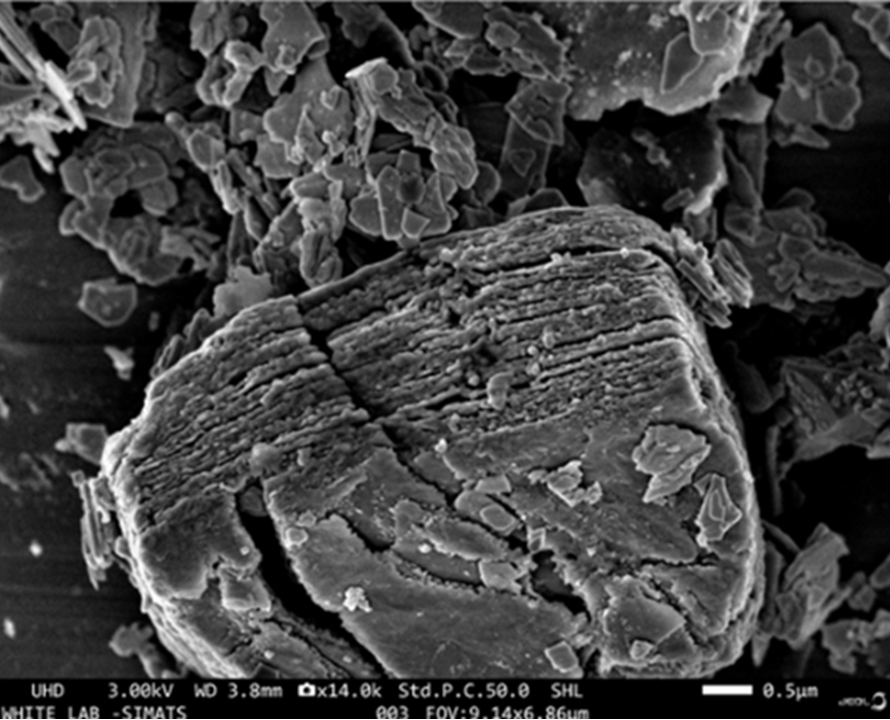
The energy-dispersive X-ray spectroscopy (EDS) analysis of Ti₃C₂–BiWO₄ nanocomposites, as shown in the Figure 2, provides crucial insights into the elemental composition and successful formation of the hybrid nanomaterial. The EDS spectrum clearly reveals the presence of bismuth (Bi), oxygen (O), carbon (C), and titanium (Ti), which are the primary constituents of the Ti₃C₂–BiWO₄ nanocomposite. The dominant peak corresponds to Bi, which exhibits the highest weight percentage (53.4%), indicating that BiWO₄ is present in a significant proportion within the composite. This is consistent with the expected stoichiometry of BiWO₄, where bismuth serves as a heavy element that contributes to its high atomic weight and prominent X-ray signal. Oxygen, with a weight percentage of 26.3%, reflects its role as a constituent of both BiWO₄ and surface-oxidized MXene, contributing to the oxide framework and surface functionalities, respectively. Carbon, detected at 16.2%, originates from the Ti₃C₂ MXene structure, affirming the presence of the carbon layers derived from the MAX phase after etching. Titanium is detected at 4.2%, confirming the successful etching of Al from the MAX phase and the presence of residual Ti in the MXene sheets. The elemental mapping and distribution confirm the effective integration of BiWO₄ onto the MXene surface without any additional impurity peaks, which suggests a uniform composite formation. The elemental ratios and absence of foreign elements validate the purity of the synthesized material. The dominant peak for Bi, with a weight percentage of 53.4%, suggests that BiWO₄ constitutes a major portion of the composite material. Oxygen, present at 26.3%, corresponds not only to the BiWO₄ lattice but also to possible surface-adsorbed species and functional groups on Ti₃C₂. Carbon and titanium, detected at 16.2% and 4.2% respectively, are key signatures of the Ti₃C₂ MXene layers, verifying their retention after the hydrothermal synthesis process. The absence of any foreign or unexpected peaks highlights the purity of the nanocomposite and confirms that no unwanted impurities were introduced during synthesis. This composition aligns well with previous EDS findings reported for MXene-based Bi-compound hybrids. The homogeneous distribution of elements across the scanned region also suggests strong interfacial interaction between the BiWO₄ and Ti₃C₂ components, crucial for enhancing biological functions such as antimicrobial and cytotoxic activity. Thus, the EDS analysis strongly supports the structural and chemical integrity of the synthesized Ti₃C₂–BiWO₄ nanocomposite system[(Akbari et al., 2024; Tamhane et al., 2024)](https://paperpile.com/c/3WztoA/IWX0+tJBV).



**Figure 2:** EDS Analysis of Ti₃C₂–BiWO₄ nanocomposite

# SEM Analysis

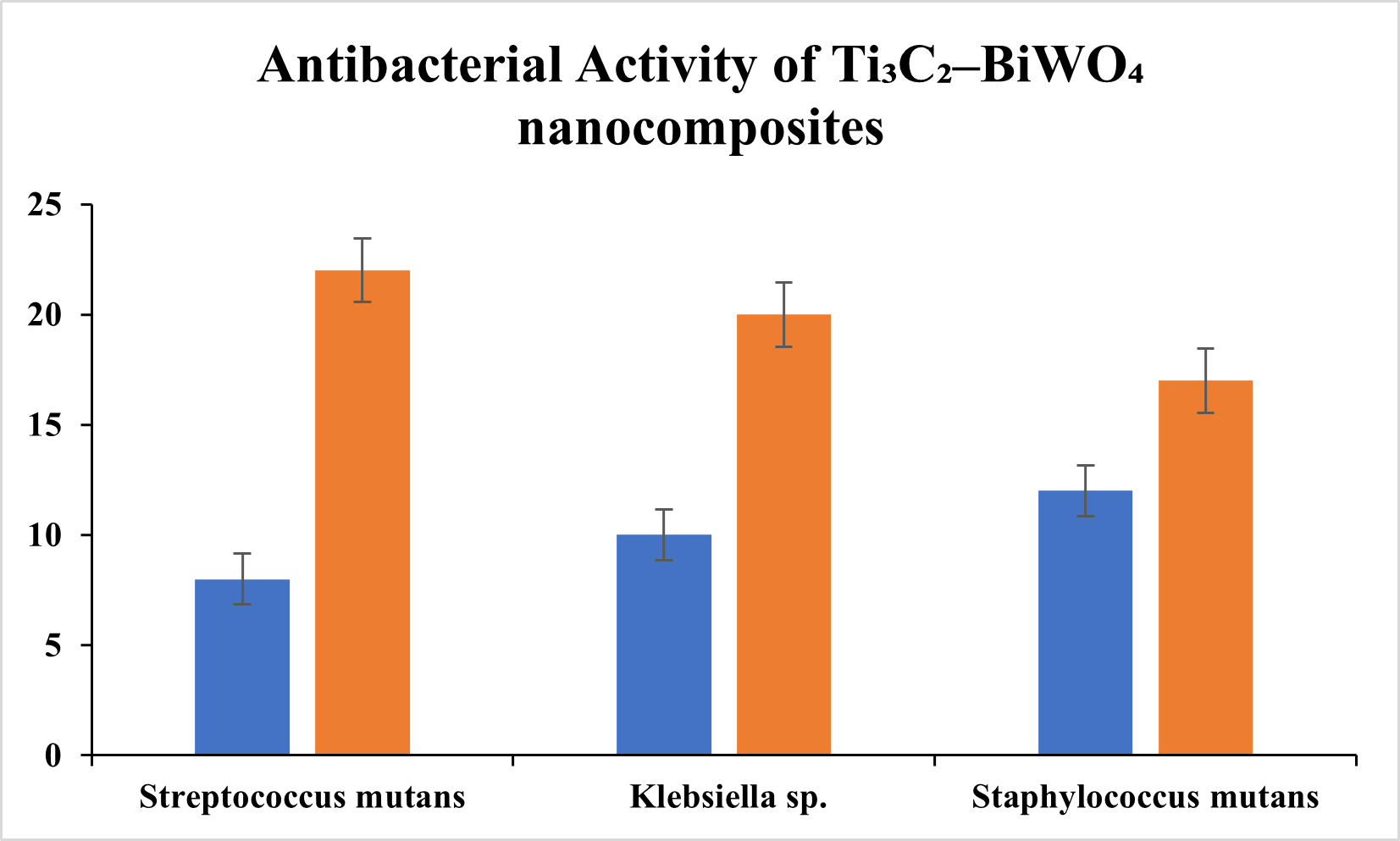
The scanning electron microscopy (SEM) image of the Ti₃C₂–BiWO₄ nanocomposite in Figure 3 clearly shows a multilayered sheet-like structure characteristic of Ti₃C₂ (a type of MXene), which displays a stacked and layered morphology with rough and uneven surfaces. These layers exhibit a crumpled and accordion-like architecture, indicative of successful exfoliation and preservation of the two-dimensional structure of Ti₃C₂ after synthesis. Overlaid on these layered nanosheets are numerous smaller BiWO₄ nanocrystals, which appear as dispersed, flaky, and irregularly shaped particles across the MXene sheets. This distribution suggests effective anchoring of the BiWO₄ nanoparticles onto the Ti₃C₂ surfaces, which is essential for creating heterojunction interfaces that enhance charge transfer and photocatalytic activity. The intimate contact between the Ti₃C₂ layers and BiWO₄ nanoparticles indicates good interaction between the two phases, which is beneficial for synergistic electronic interactions and suppressing charge recombination. The high surface area and open structure visible in the SEM image support the idea that the nanocomposite offers sufficient active sites for interaction with biological or environmental substrates. Additionally, the presence of rough edges and sharp contours on the particles enhances the nanocomposite’s reactivity by exposing more active sites. The scale bar of 0.5 µm and the magnification of ×14,000 affirm the nanoscale features and fine dispersion of BiWO₄ on the Ti₃C₂ scaffold. The SEM analysis of Ti₃C₂–BiWO₄ nanocomposites reveals a distinct microstructural morphology, characterized by layered, sheet-like Ti₃C₂ structures interspersed with irregularly shaped BiWO₄ nanoparticles. The Ti₃C₂ nanosheets exhibit a wrinkled and folded architecture, which is a typical feature of delaminated MXene layers, providing a high surface area and accessible interlayer spacing for nanoparticle deposition. BiWO₄ particles appear uniformly anchored onto the Ti₃C₂ matrix, suggesting effective integration between the two phases. This spatial distribution not only supports strong interfacial contact but also plays a vital role in improving charge separation efficiency and (Nikalje et al., 2024) (Chehelgerdi et al., 2023)photocatalytic performance. The rough and porous surface evident in the SEM image allows greater active site exposure, which is crucial for applications like phototherapy and catalysis. The hierarchical morphology formed by the combination of lamellar Ti₃C₂ and granular BiWO₄ ensures synergistic effects, enabling enhanced electron transport and reduced recombination rates[(Lakshmi Anvitha et al., 2024; Liu et al., 2020)](https://paperpile.com/c/3WztoA/76Tv+Wu7E).



**Figure 3:** SEM Analysis of Ti₃C₂–BiWO₄ nanocomposites

# Antibacterial Activity

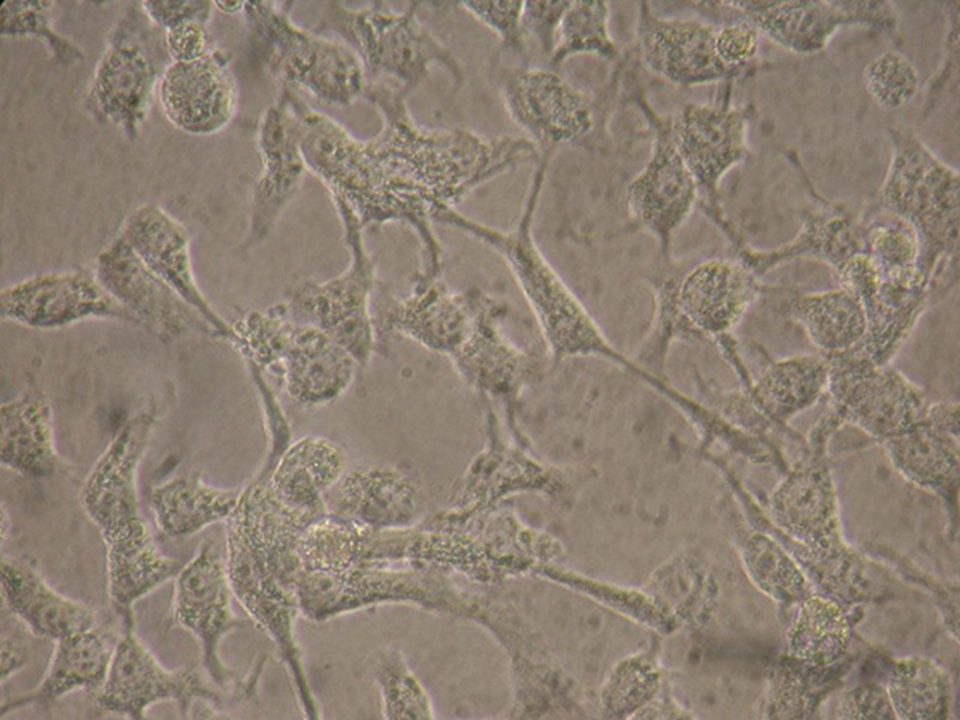
The antibacterial activity exhibited by Ti₃C₂–BiWO₄ nanoparticles against both Gram-positive The antibacterial potential of Ti₃C₂–BiWO₄ nanopaArticles was evaluated against both Gram-positive (*Streptococcus mutans* and *Staphylococcus mutans*) and Gram-negative (*Klebsiella* sp.) bacteria in Figure 4 (Graph 1). The study employed two concentrations of Ti₃C₂–BiWO₄ nanoparticles (10 µg/mL and 20 µg/mL) to assess their effectiveness in inhibiting bacterial growth by measuring the zone of inhibition. The results demonstrated that Ti₃C₂–BiWO₄ nanoparticles possess notable antibacterial activity against all tested strains, although their effectiveness varied depending on the bacterial species and nanoparticle concentration. At a concentration of 10 µg/mL, the nanoparticles produced inhibition zones of 8 mm for *Streptococcus mutans*, 10 mm for *Klebsiella* sp., and 12 mm for *Staphylococcus mutans*, indicating a moderate antibacterial effect. For comparison, tetracycline, used as a positive control at a concentration of 20 µg/disc, showed significantly higher antibacterial activity, with inhibition zones measuring 24 mm, 23 mm, and 17 mm for *Streptococcus mutans*, *Klebsiella* sp., and *Staphylococcus mutans*, respectively. While the antibacterial effect of Ti₃C₂–BiWO₄ nanoparticles was lower than that of tetracycline, the results still suggest promising potential for these nanomaterials in antibacterial applications, particularly due to their broad-spectrum activity against both Gram-positive and Gram-negative bacteria, indicates that their antimicrobial efficacy may be enhanced by increasing the concentration, offering a foundation for further studies focused on dose optimization and mechanistic analysis. and Gram-negative bacteria highlights their potential as effective antimicrobial agents. The observed inhibition zones, although moderate compared to the positive control tetracycline, demonstrate a clear capacity of the nanocomposites to suppress microbial growth. The differences in inhibition zones among the bacterial strains may be attributed to variations in cell wall structures, with Gram-negative bacteria like *Klebsiella* sp. generally being more resistant due to their outer membrane barrier[(Silhavy et al., 2010)](https://paperpile.com/c/3WztoA/HzOE). Nonetheless, Ti₃C₂–BiWO₄ still showed effective action against *Klebsiella* sp., suggesting that the nanocomposites can interact with or penetrate bacterial membranes to exert their effects. The antimicrobial mechanism may involve the generation of reactive oxygen species (ROS) or disruption of membrane integrity, as reported in similar studies involving Bi-based and MXene-based materials[(Rasool et al., 2016)](https://paperpile.com/c/3WztoA/BKqI). While the zones of inhibition for Ti₃C₂–BiWO₄ at 10 µg/mL were lower than those of tetracycline, increasing the concentration could enhance efficacy, supporting its potential as a complementary or alternative antimicrobial treatment. These findings contribute to the growing body of research on nanomaterials as promising candidates in the fight against antibiotic-resistant pathogens.[(S. Kumar et al., 2024)](https://paperpile.com/c/3WztoA/XV2F)



**Figure 4:** Antibacterial activity of Ti₃C₂–BiWO₄ nanocomposites against *Streptococcus mutans, Klebsiella sp.,* and *Staphylococcus mutans*

## Cytotoxic Activity

The cytotoxic effect of Ti₃C₂–BiWO₄ nanocomposites on HCT116 colorectal cancer cells was evaluated using microscopic observation in figure 5, revealing clear morphological changes indicative of cellular stress and apoptosis. In the provided micrograph, cells exposed to the nanocomposite exhibit significant alterations in shape, detachment from the culture surface, and shrinkage, which are characteristic features of cytotoxic damage. Compared to untreated cells that typically display a flattened, elongated morphology with clear cell-to-cell contact, the treated HCT116 cells show a loss of membrane integrity, increased granularity, and fragmented structures, suggesting the onset of apoptotic pathways. These findings imply that the Ti₃C₂–BiWO₄ nanocomposite exerts potent cytotoxic activity, likely through the generation of reactive oxygen species (ROS) and disruption of mitochondrial function, which are common mechanisms in nanomaterial-induced cell death [(Singh et al., 2024; Vohra et al., 2024)](https://paperpile.com/c/3WztoA/tOUZ+x5B2). The layered structure of Ti₃C₂, a MXene material, offers high surface reactivity, while the incorporation of BiWO₄ enhances photocatalytic properties, potentially leading to oxidative stress within cancer cells. Such stress could activate caspase-mediated apoptosis or interfere with cell cycle progression, ultimately resulting in decreased cell viability [(Keerthana & Ramesh, 2021; Murugesan, 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/3WztoA/atI6q+QKThX+TuGR5)[(Keerthana & Ramesh, 2021; Murugesan, 2021; Subramanian et al., 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/3WztoA/atI6q+QKThX+TuGR5+serHK). The cytotoxic activity of Ti₃C₂–BiWO₄ nanocomposites against HCT116 colorectal cancer cells demonstrates notable anticancer potential, as evidenced by morphological changes such as cellular shrinkage, membrane blebbing, and detachment from the substrate following treatment [(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/3WztoA/q3BkH+EHIbK+WoElV) These changes are indicative of apoptosis and cellular stress, aligning with previous studies on MXene-based nanomaterials that exhibit high surface reactivity and ROS generation capabilities[(Okada et al., 2019)](https://paperpile.com/c/3WztoA/gyUR). The synergistic combination of Ti₃C₂, known for its excellent electron conductivity and layered structure, with BiWO₄, a visible-light-responsive photocatalyst, enhances oxidative stress in cancer cells, leading to mitochondrial disruption and apoptosis [(G. & Ganapathy, 2022; I. L. Kumar & Ramesh, 2021)](https://paperpile.com/c/3WztoA/Xah7f+kx6lS)). The photoactive nature of BiWO₄ may further intensify intracellular ROS levels under physiological conditions, damaging DNA and proteins critical for cancer cell survival. These effects likely activate caspase-mediated apoptotic pathways and impair cell cycle progression. The observed morphological responses in HCT116 cells are consistent with literature demonstrating nanomaterial-induced apoptosis through oxidative and mitochondrial stress mechanisms[(B et al., 2024)](https://paperpile.com/c/3WztoA/YVPf).



**Figure 5:** Cytotoxic Activity of Ti₃C₂–BiWO₄ nanocomposites

# CONCLUSION

The present study successfully demonstrates the hydrothermal synthesis of Ti₃C₂–BiWO₄ nanocomposites and their potential for antimicrobial and cytotoxic applications. X-ray diffraction (XRD) analysis confirmed the formation of a crystalline nanocomposite structure with distinct peaks corresponding to both Ti₃C₂ MXene and BiWO₄, indicating successful integration of the two components without compromising their individual crystalline identities. Scanning electron microscopy (SEM) images revealed the layered morphology of Ti₃C₂ decorated with BiWO₄ nanoparticles, highlighting a high surface area and well-dispersed structure that facilitates effective microbial contact and cellular interaction. Energy-dispersive X-ray spectroscopy (EDS) analysis confirmed the elemental composition of the nanocomposite, showing the presence of Ti, Bi, W, O, and C, further validating the successful synthesis of Ti₃C₂–BiWO₄. Antimicrobial assessments demonstrated significant inhibitory effects against both fungal species (*Candida albicans* and *Candida parapsilosis*) and bacterial strains (*Enterococcus faecalis* and *Streptococcus mutans*), with enhanced activity observed at 100% concentration, particularly notable in the zone of inhibition measurements. These effects are attributed to the synergistic action of Ti₃C₂’s sharp edges and BiWO₄’s photocatalytic ROS generation, which disrupt microbial membranes. Furthermore, the cytotoxic evaluation against HCT116 colorectal cancer cells revealed pronounced morphological alterations characteristic of apoptosis, suggesting that the nanocomposite induces oxidative stress and interferes with cellular homeostasis. Collectively, these findings indicate that the Ti₃C₂–BiWO₄ nanocomposites synthesized via a hydrothermal route offer a multifunctional platform with strong antimicrobial efficacy and potential anticancer capabilities, making them promising candidates for biomedical applications, especially in antimicrobial coatings and targeted cancer therapies.

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