

Antimicrobial Evaluation of WO₃–TiO₂ Nanocomposites Against Pathogenic Bacterial and Fungal Strains.

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ABSTRACT

In this study, WO₃/TiO₂ nanocomposites were synthesized and thermally calcined at 800°C and 1200°C to evaluate the effect of calcination on structural, optical and antimicrobial performance. XRD and FTIR confirmed the formation of mixed-phase crystalline frameworks, while UV-Vis spectroscopy revealed strong visible-light absorption and Tauc plot analysis indicated reduced band gap energies, enhancing antibacterial and antifungal activity. HR-SEM micrograph showed uniformly dispersion spherical nanoparticles with narrow size distribution and minimal agglomeration. The antimicrobial activity of WO₃, TiO₂, and their WO₃/TiO₂ nanocomposites was evaluated against both bacterial and fungal strains. The WO₃/TiO₂ nanocomposites exhibited excellent antibacterial and antifungal activities, with the sample calcined at 1200°C showing significantly higher inhibition zones than the 800°C sample against both bacterial and fungal pathogens. *Candida albicans* was more susceptible than *Aspergillus niger*, while strong antibacterial activity was observed against both Gram-positive and Gram-negative bacteria. The enhanced antimicrobial performance is attributed to improved crystallinity, efficient interfacial charge transfer, and increased reactive oxygen species (ROS) generation, resulting in effective disruption of microbial cell membranes. These findings demonstrate that WO₃/TiO₂ nanocomposites are promising eco-friendly materials for antimicrobial coatings, environmental disinfection, and water purification applications.

Keywords: WO₃/TiO₂ Nanocomposites, HR-SEM, Antibacterial activity, Gram-positive, Gram-negative

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1. Introduction

Nanomaterials have attracted great attention owing to their high surface-to-volume ratio, quantum confinement effects, and tunable physicochemical properties. These features make them promising for application in catalysis, energy conversion, environmental purification, and healthcare. Among them, nanocomposites engineered by integrating multiple material at the nanoscale are particularly promising as they exhibit synergistic effects that enhance photocatalysis, antimicrobial treatment and environmental performance [1-6].

The emergence of antibiotic resistant bacteria has created an urgent need for alternative antimicrobial strategies. Coupling semiconductors with complementary band alignment has proven effective in enhancing antibacterial activity by facilitating electron-hole separation, suppressing recombination, and promoting interfacial charge transfers [7-11]. This process enables efficient generation of reactive oxygen species (ROS) which play crucial role in microbial inactivation under light irradiation [12-14]. Several semiconductor combination such as TiO₂–WO₃, WO₃–SiO₂ [16–17], TiO₂–CeO₂ [20], TiO₂–Fe₂O₃ [21], and WO₃–BiVO₄ [26–27], have been reported for antibacterial application. TiO₂ and WO₃ are especially notable due to their photoactivity, durability, chemical stability, and large surface area [28-29].

In this study, WO₃/TiO₂ nanocomposites calcined at 800°C and 1200°C were investigated against *Streptococcus mutans*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*. Unlike prior works focussing on *Staphylococcus aureus* and *Klebsiella pneumoniae* [36-39], this study emphasizes *S. mutans* and *S. typhi* show enhanced antibacterial activity, particularly at 1200°C attributed to improved crystalline and ROS mediated bacterial inactivation [40-42].

2. Synthesis

All the chemicals of AR grade were purchased from Sigma Aldrich and used without further purification.

2.1 Preparation of WO₃ & TiO₂ nanoparticles

This **Figure 1** shows the flow chart for the synthesis of WO₃ nanoparticles by the co-precipitation method and TiO₂ nanoparticles by the hydrothermal method, outlining the step-by-step procedures involved in each process

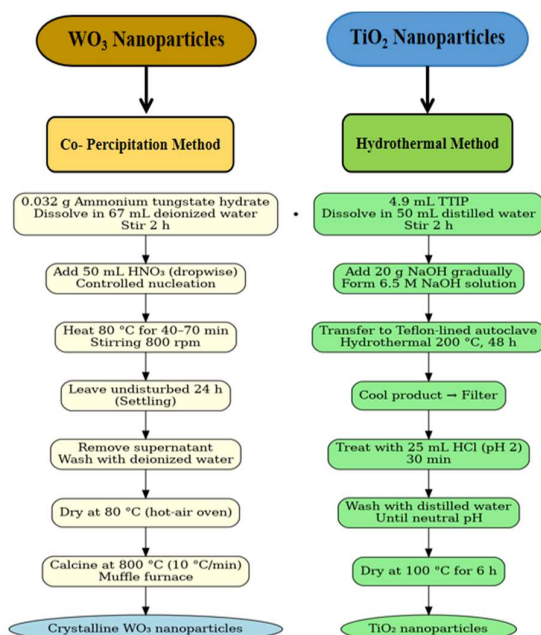


Figure 1: Flow chart for the synthesis of WO₃ and TiO₂ nanoparticles using the co-precipitation and hydrothermal methods.

2.3 Preparation of WO₃/TiO₂ Nanocomposites

The synthesis of WO₃/TiO₂ nanocomposite involved the following steps, following the method described by Pined-Escobar, J.A. [38]:

Step 1: Titanium isopropoxide (4.5 ml) was mixed with 50 ml of 2-propanol and 5 ml of acetic acid, ensuring uniform dispersion in an acidic medium.

Step 2: Separately, 1 g of WO₃ and 1 g of TiO₂ were dispersed in 40 ml of 2-propanol and ultrasonicated for 30 min using an ultrasonic probe (VXC 130, Sonics & Materials, Inc.) to form a homogeneous suspension, which was then slowly introduced into the acidic titanium solution.

Step 3: Hydrolysis was initiated by adding 5 ml deionized water dropwise, forming a gel-like network maintained at 70 °C for 60 mins under stirring and reflux conditions.

Step 4: The obtained WO₃/TiO₂ samples were dried at 100 °C for 24 hrs to eliminate residual solvent and moisture.

Step 5: The dried powders were calcined at 800 °C and 1200 °C for 4 h in air. Under light irradiation, WO₃/TiO₂ generates reactive oxygen species that disrupt bacterial membranes, denature proteins, fragment DNA, and inhibit replication, ensuring potent antibacterial activity.

3. Characterizations

Powder X-ray diffraction (XRD) analysis was performed using a Bruker D8 Advance instrument with Cu K α radiation ($\lambda = 1.5416$ Å). The optical bandgap was assessed using an Ocean Optics UV-Vis spectrophotometer, while functional group analysis was conducted with a Perkins Elmer

Spectrum 2 Fourier Transform Infrared (FTIR) spectrometer. High-resolution scanning electron microscopy (HRSEM) combined with energy-dispersive X-ray (EDX) analysis was carried out on an FEI Quanta 200 instrument, operated at 25 kV, to examine the morphology and elemental composition of the prepared powders [39].

4. Antibacterial Assay

The antibacterial evaluation of the WO₃/TiO₂ nanocomposites heat-treated at 800 °C and 1200 °C was conducted using disc diffusion and Muller-Hinton Agar (MHA) methods. The study focused on both Gram-positive strains, namely *Streptococcus mutans* and *Bacillus subtilis*, as well as Gram-negative strains, specifically *Escherichia coli* and *Salmonella typhi*. The methodologies employed allowed for the measurement of the inhibition zones around the nanocomposite discs, providing quantitative data on the antibacterial efficacy of the materials. Further insights into the antibacterial performance and its relevance are provided in the Results and Discussion section.

5. Results and Discussion

5.1 Powder XRD Analysis

Figure (2a-2d) presents the X-ray diffraction (XRD) patterns of WO₃, TiO₂, and WO₃/TiO₂ nanocomposites calcined at 800 °C and 1200 °C. The WO₃ sample (**Figure 2a**) is crystalline in the monoclinic phase, as confirmed by diffraction peaks matching JCPDS No. 083-0950, with prominent reflection at 2θ values of 23.1°, 23.5°, 24.3°, 33.2°, 41.4°, 49.9°, and 50.3°, indexed to the (002), (020), (200), (022), (202), (222), (400), and (114) planes, respectively. The calculated lattice parameters ($a = 7.613$ Å, $b = 7.455$ Å, $c = 7.269$ Å) align well with reported monoclinic WO₃ structures [30]. Similarly, the TiO₂ sample (**Figure 2b**) exhibits distinct peaks consistent with the rutile phase, verified by JCPDS No. 75-1537. The diffraction maxima observed at $2\theta = 27.4^\circ, 36.0^\circ, 39.2^\circ, 41.2^\circ, 44.0^\circ, 54.3^\circ, 56.6^\circ, 62.7^\circ, 64.0^\circ, 65.3^\circ,$ and 69.0° correspond to the (110), (101), (200), (111), (210), (211), (220), (002), (310), (221), and (301) planes, and the rutile TiO₂ phase exhibits lattice constants of $a = 4.565$ Å, $b = 4.568$ Å, and $c = 3.776$ Å. Crystallite size calculated using the Scherrer equation were 42 nm for WO₃ and 87 nm for TiO₂ confirming the formation of well-defined crystalline structure. For the WO₃/TiO₂ nanocomposites (**Figures 2c-2d**), the XRD pattern confirms the coexistence of rutile TiO₂ and monoclinic WO₃ phases without detectable impurities, exhibiting > 90% crystallinity. Such high structure integrity is essential for antibacterial activity [40-41], as the stable, well-crystalline, and high-surface-area structure promotes efficient ROS generation and enhances interaction with bacterial cells.

The structure insight provided by XRD is further supported by the WO₃/TiO₂ and WO₃/TiO₂ nanocomposites recorded in the 400- 4000 cm⁻¹ range **Figure (2e-2h)**. For WO₃ broad band at 3370 cm⁻¹ corresponds to O-H stretching vibrations,

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while peaks at 1615 cm⁻¹ and 1406 cm⁻¹ are assigned to H-O-H bending of adsorbed water [43] and W-O-H vibration confirming surface hydroxyl and water interaction. In TiO₂ O-H stretching appears at 3374 cm⁻¹, the water bending mode is observed at 1640 cm⁻¹ and characteristics band at 481 cm⁻¹ corresponds to Ti-O bending and Ti-O vibration, consistent with the structural stability revealed by XRD [44-46]. The WO₃/TiO₂ nanocomposites display combined vibrational modes of both oxides with a strong O-H stretching band at 2965 cm⁻¹ confirming retention of hydroxyl groups a band at 2300 cm⁻¹ attributed to atmospheric CO₂ and a deformation band at 1632 cm⁻¹ confirming adsorbed water [47]. Importantly a strong W-O-W stretching vibration at 941 cm⁻¹ observed in sample calcined at 800°C and 1200°C verifies the incorporation and stabilization of WO₃ within the composites. Together, the XRD and FTIR analyses that the WO₃/TiO₂ nanocomposites possess high crystallinity, stable lattice frameworks and preserved functional groups (hydroxyl, Ti-O, and W-O-W bonds), which not only ensure structural stability but also provide active surface sites for ROS generation. These synergistic structural and vibrational features strongly support the potential of WO₃/TiO₂ nanocomposites as efficient antibacterial materials.

The optical properties of WO₃, TiO₂ and WO₃/TiO₂ nanocomposites were systematically examined using UV-Vis absorption spectroscopy in the wavelength range of 250 - 800 nm, as presented in (Figures 3a-3d). WO₃ displayed a prominent absorption peak at 470 nm, TiO₂ at 495 nm, while the WO₃/TiO₂ nanocomposites calcined at 800°C and 1200°C exhibited peaks at 400 nm and 454 nm, respectively. These absorption features demonstrate the ability of the material to utilize visible light effectively, which is crucial for driving antibacterial processes.

The band gap energies of pure WO₃ and TiO₂ were estimated using the relation

$$E_g = 1240/\lambda$$

Where λ represents the absorption edge. Based on

the observed spectra, the bandgap value were calculated as 2.53 eV for WO₃ (470 nm) and 2.7 eV for TiO₂ (495 nm). These values align well with the intrinsic semiconductor properties of the two oxides and confirms their capability as visible-light responsive antibacterial activity [48]. To further understand the electronic behavior of WO₃/TiO₂ composites, the Tauc plot methods was employed, applying the equation.

$$(\alpha h\nu)^n = A(h\nu - E_g)$$

Where α is the absorption coefficient, $h\nu$

is photon energy, and E_g is the bandgap energy [48-49]. From this analysis, the composites calcined at 800°C and 1200°C were found to possess bandgap of 3.0 eV and 3.15 eV, respectively.

Notable, the slight increase in bandgap with higher calcination temperature is associated with improved photon absorption, better charge separation, and reduced electron-hole recombination. These properties directly enhance ROS production, which induces lipid peroxidation, protein denaturation, and DNA fragmentation in bacterial. Consequently, WO₃/TiO₂ nanocomposites, especially at 1200°C exhibit superior antibacterial activity through efficient ROS-mediated disinfection.

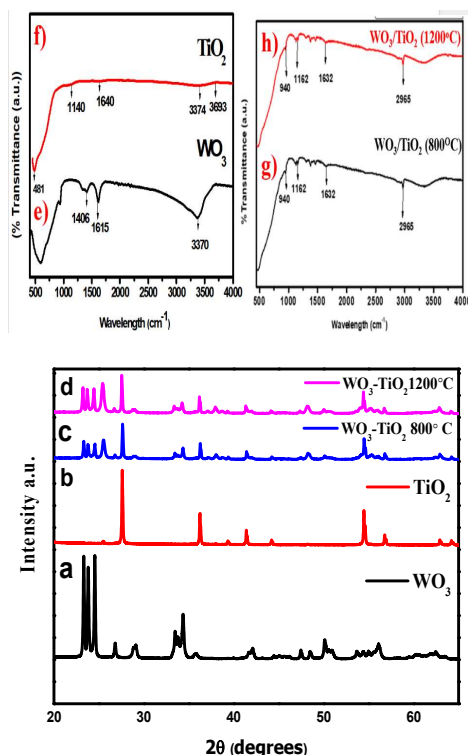
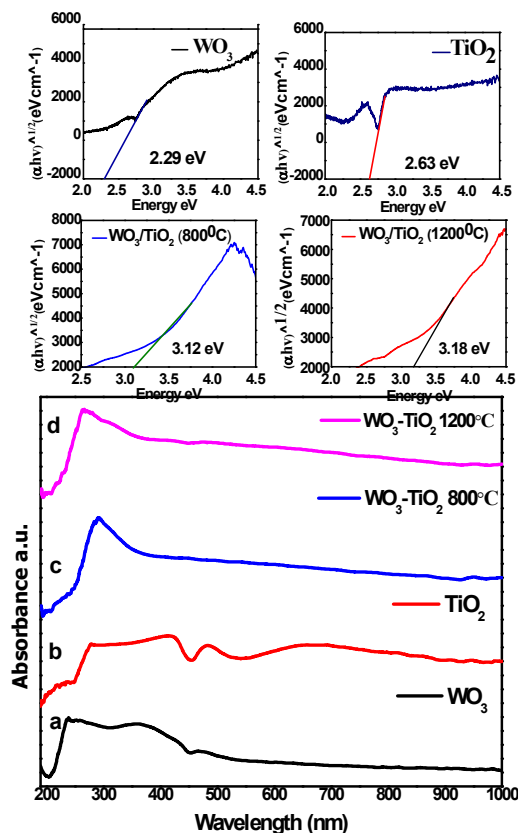


Figure 2. (a-d) XRD patterns of WO₃, TiO₂, and WO₃-TiO₂ nanocomposites calcined at 800 °C and 1200 °C, and (e-h) FTIR spectra of the same samples.

5.2 UV-Visible Analysis

Figure 3: UV- Visible spectroscopy for (a) WO₃, (b)



TiO₂ and (c) WO₃-TiO₂ (800 °C) and (d) WO₃-TiO₂ (1200°C).

5.3 HR-SEM Analysis

Figure 4(a-h) presents the High-Resolution Scanning Electron Microscopy (HR-SEM) images and Energy Dispersive X-ray (EDX) spectra of pure WO₃, TiO₂, and WO₃/TiO₂ nanocomposites subjected to thermal treatment. The HR-SEM micrograph (**Figure 4a-4d**) clearly indicate that the particles exhibit a range of sizes with noticeable agglomeration, which is typical for nanoparticle system due to their high surface energy. Pure WO₃ (**Figure 4a**) appears as regular nanoparticles with approximate dimension of 500 nm in length and width, forming a relatively uniform morphology. In contrast, the HR-SEM images of WO₃/TiO₂ nanocomposites (**Figure 4b-4d**) reveals nearly spherical nanoparticles with an average diameter of 31 nm. The nanocomposites are densely packed and interconnected, with increased agglomeration attributes to high temperature calcination at 800°C and 1200°C. This thermal treatment promotes grain growth, densification, and reduced porosity, which collectively enhance surface area and facilitate improved charge transfers factor crucial for antibacterial activity. These structure features strengthen surface interaction and ROS generation, thereby improving antibacterial performances.

The corresponding EDX spectra (**Figure 4e-4h**) further confirm the successful synthesis and compositional purity of the samples. The spectra clearly identify the characteristic elemental peaks of tungsten (W), titanium (Ti), and oxygen (O), which are uniformly distributed across the nanocomposites. The absence of foreign element such as carbon or other contaminants highlight the high purity of the synthesized WO₃/TiO₂ composites. Moreover, the EDX spectra of nanocomposites treated at 800°C and 1200°C (**Figure 4g-4h**) display no significant peak shift, indicating that the thermal treatment maintains structural integrity while promoting close interfacial contact between WO₃ and TiO₂. Thus, the combined HR-SEM and EDX analyses provide critical insight into the optimized morphology composition, and purity of WO₃/TiO₂ nanocomposites, underscore their potential for biological and environmental applications.

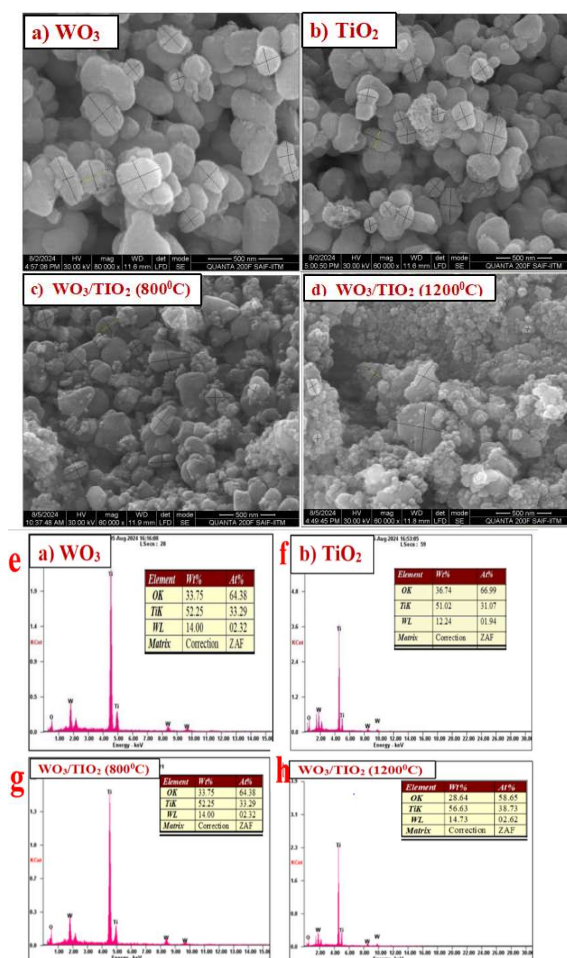


Figure 4: HRSEM analysis (a) WO₃ (b) TiO₂ (c) WO₃-TiO₂ (800 °C), (d)WO₃-TiO₂ (1200°C) Figure 6: EDX Spectra (e) WO₃, (f) TiO₂, (g) WO₃-TiO₂ (800°C), (h) WO₃-TiO₂ (1200°C),

6. Antibacterial Activity Evaluation

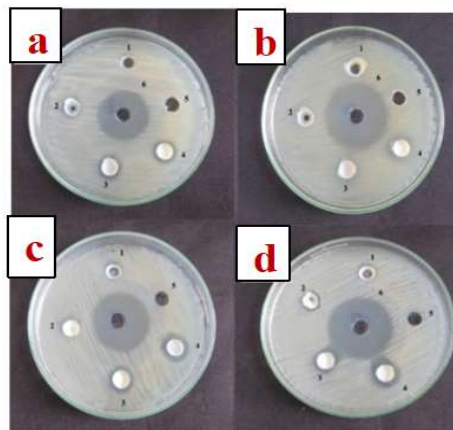
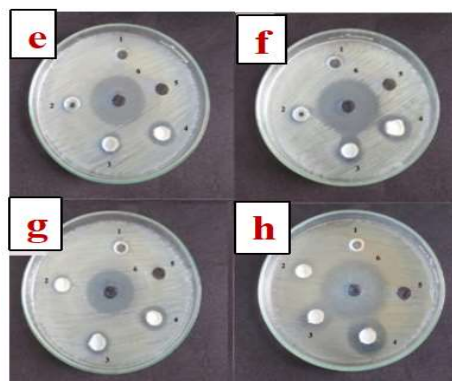
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The antibacterial activity of WO₃/TiO₂ nanocomposites was evaluated using the agar diffusion methods on Muller-Hinton Agar (MHA) medium againsts Gram-positive (*Streptococcus mutans*, *Bacillus subtilis*) and Gram-negative (*Echerichia coli*, *Salmonella typhi*) bacteria, with streptomycin as the positive control. MHA was prepared by dissolving 3.8 g of MHA and 2g of agar in 100 ml of distilled water, sterilized, and poured into petri plates. Nanocomposites were serially diluted in nutrient broth, inoculated with bacterial cultures, are incubated for 24 h at 37°C to determine minimum inhibitory concentration (MIC). Using DMSO as solvent, four concentration (125, 250, 500, and 1000 µl) were tested, while streptomycin (1 mg/ml, 20 µl) as the positive control. The lowest concentration suppressing bacterial growth was noted as the MIC.

Nanocomposites synthesized at 1200°C displayed greater antibacterial compared to those synthesized at 800 °C. Inhibition zones expanded from 10-14 mm (800 °C) to 14-20 mm (1200 °C), with *S.typhi* showing the largest increase. Control inhibition zones measured 30-36 mm (800 °C) and 31-36 mm (1200 °C). Enhanced activity at 1200 °C is attributed to improved crystalline, phase formation, and increased ROS generation, leading to stronger bacterial membranes disruption and lower MIC values. These result confirm that higher synthesis temperatures improve antibacterial performance, highlighting WO₃/TiO₂ nanocomposites as promising candidates for medical, environmental and industrial application. **Table 1** and **Figure 5** present detailed inhibition zone comparisons. **Table 1:** Comparison of WO₃/TiO₂ nanocomposites for antibacterial activity

Microorganism	Zone of inhibition in mm					References
	Volumes (µl/ml)				Streptomycin (control)/ Ampicillin	
	125	150	500	1000		
<i>Staphylococcus aureus</i>			10	19	27	[51]
<i>Bacillus subtilis</i>	-	-	10	14	20	[52]
<i>E.coli</i>	-	-	10	16	20	
<i>Salmonella typhi</i>			8	14	19	
<i>E.coli</i>	-	10	11	16	20	[53]
<i>Bacillus subtilis</i>	-	-	9	12	20	[54]
<i>Salmonella typhi</i>			8	14	10	
<i>E.coil</i>	-	10	11	12	16	[55]
<i>E.coli</i> , <i>K.pneumoniae</i> , <i>S. aureus</i> , <i>P. Vulgaris</i> ,and <i>P.aeruginosa</i>	-	10	11	12	19	[56]

<i>E. coli</i>	-	9	10	14	16	[57]
<i>Staphylococcus Mutants (800°C)</i>	9	11	12	13	31	This work
<i>E.coil (800°C)</i>	8	11	13	15	36	This work
<i>Salmonella typhi (800°C)</i>	0	10	12	14	33	This work
<i>Bacillus subtilis (800°C)</i>	10	11	12	15	36	This work
<i>S. Mutants (1200°C)</i>	10	11	13	15	31	This work
<i>B.Subtilis (1200°C)</i>	10	12	14	18	36	This work
<i>E. coli (1200°C)</i>	10	12	13	17	30	This work
<i>Salmonella typhi (1200°C)</i>	-	13	15	20	35	This work



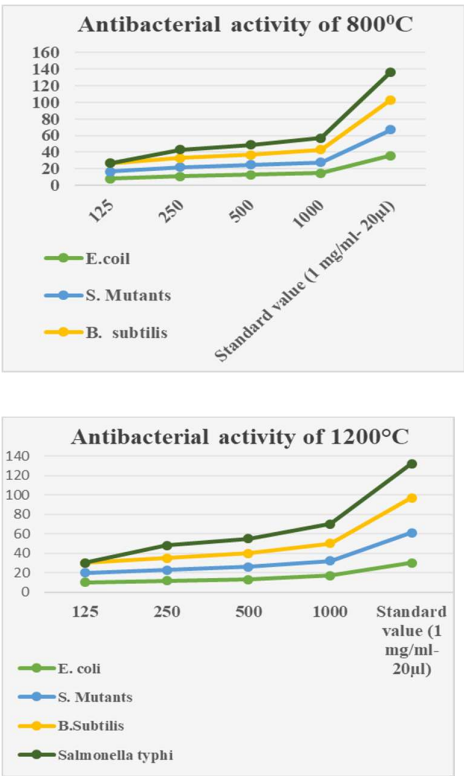
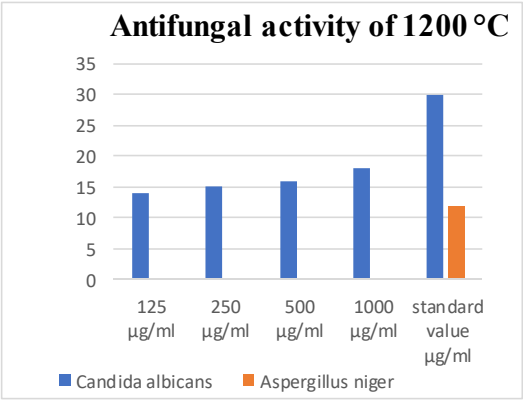


Figure 5: Antibacterial activity of WO₃-TiO₂ nanocomposites calcined at 800 °C against (a) *S. mutans*, (b) *B. subtilis*, (c) *E. coli*, and (d) *S. typhi*, and at 1200 °C against (e) *S. mutans*, (f) *B. subtilis*, (g) *E. coli*, and (h) *S. typhi*, along with (i) schematic flow chart illustrating the antibacterial mechanism of WO₃-TiO₂ (800–1200 °C) nanocomposites.

7. ANTIFUNGAL ACTIVITY

In general, the WO₃ and TiO₂ nanocomposites effectively prevent fungal growth, break biofilms and deactivate spores. They generate reactive oxygen species (ROS) that damage critical biological components such as membranes, protein, lipids, and nucleic acids, leading to fungal cell death or growth retardation. Furthermore, the capacity to dissolve biofilms structures increases treatment efficacy and inhibits spore germination, making these nanocomposites especially beneficial against drug-resistant fungal strains [39-40]. The co-precipitate synthesized WO₃/TiO₂ nanocomposites at 800°C and 1200°C were subjected to antifungal treatment against *Candida albicans* and *Aspergillus niger* using the zone of inhibition (ZOI) methods [41]. Figure 6 show the degree of the antifungal activity in the clear zones surrounding the sample wells. Table 1 presents a quantitative summary of the result obtained. Table 1: literature survey of WO₃/TiO₂ Nanocomposites for antifungal activity

Microorganism	Zone inhibition nm					References
	100 µg	200 µg	400 µg	600 µg	Standard (1mg/ml-20 µl)	
Aspergillus niger-MTCC-282 TC	0	11 ± 0.40	23 ± 0.25	19 ± 0.41	32 ± 0.25	
Aspergillus niger-MTCC-282 W 0.75 h	0	15 ± 0.25	28 ± 0.35	22 ± 0.3	32 ± 0.25	
Microorganism	150 µg	250 µg	500 µg	1000 µg	Standard (1mg/ml-20 µl)	Current Research work
Candida albicans (800 °C)	0	12	14	15	20	Current work
Aspergillus niger (800 °C)	-	-	-	-	10	
Candida albicans	14	15	16	18	30	



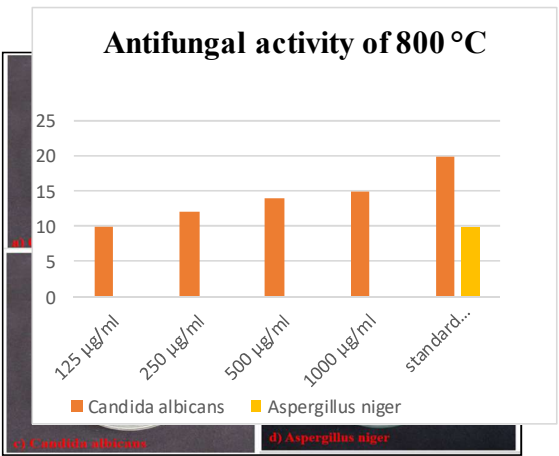
ans (1200)					
Aspergillus niger (1200)	-	-	-	-	12

From literature review, the *Aspergillus niger* (MTTCC-282 TC) had a maximal inhibition zone of (11-23nm) at 1 mg/ml-20L at lower concentration (100 µg) for control at 32 nm. However at modified condition (MTTCC-282 TC W 0.75h) there is a slight increase in inhibition was observed at 15-28 nm (37) under same concentration. In the present study, the prepared nanocomposites at 800°C reveals the moderate inhibition against *Candida albicans* (10-15 nm) whereas, inhibition against *A. niger* was ineffective. In contrast, nanocomposites synthesized at 1200°C shows better inhibitory zones (14-18 nm) for *Candida albicans* closer to standard medication efficacy and it shows moderate activity (12 nm) for *A. niger*. Perhaps, *Candida Albicans* shows greater susceptibility than *A. niger*, due to differences in cell wall composition. Compared with the previously reported data [41] the results of WO₃/TiO₂ nanocomposites synthesized at 800°C and 1200°C have higher antifungal activity. The samples prepared at 1200°C show better inhibitory zones compared with the samples at 800°C signifies the synthesis temperature has an impact on antifungal efficiency for the present work. Higher temperatures also improve ROS generation, charge separation, and structural properties, which boost membrane disruption [42- 43].

Figure 6: Antifungal performances of WO₃/TiO₂ Nanocomposites treated at 800°C and 1200°C

Figure 7: Schematic depicting the antifungal efficacy of WO₃/TiO₂ nanocomposites at 800°C and 1200°C.

8. Conclusion



WO₃/TiO₂ nanocomposites were successfully synthesized by the coprecipitation method and calcined at 800 °C and 1200 °C. XRD confirmed the formation of monoclinic WO₃ and rutile TiO₂ phases with crystallite size of 42 nm and 87 nm. HRSEM revealed spherical nanoparticles (31 nm), while EDX confirmed the uniform distribution of W, Ti, and O elements. ftir analysis showed the presence of surface hydroxyl groups, and UV- Visible studies indicated visible- light absorption with band gap energies of 3.00 eV and 3.15 eV for the composites calcined at 800°C and 1200°C respectively. The WO₃/TiO₂ nanocomposites exhibited excellent antibacterial activity against *Streptococcus mutans*, *Bacillus subtilis*, *Escherichia coli*, and *Salmonella typhi*, as well as strong antifungal activity against *Candida albicans* and *Aspergillus niger*, with the 1200°C calcined sample showing the highest inhibition. The enhanced antimicrobial performance is attributed to improved crystallinity and increased reactive oxygen species (ROS) generation, which effectively damages microbial cell membranes and intracellular components. These findings demonstrate that WO₃/TiO₂ nanocomposites are promising eco-friendly antimicrobial materials for biomedical applications, environmental disinfection, water treatment, and antimicrobial coatings

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