Nickel Oxide-g-C3N4 Nano-Composites: Revealing Improved Antifungal Activity for Clinical Application

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**Abstract:** This work describes the synthesis and antifungal assessment of nanocomposites made of nickel oxide-g-C3N4 (NiO-g-C3N4). These sol-gel-prepared nanocomposites had strong antifungal activity against Aspergillus niger and Candida albicans, as determined by XRD, SEM, and FTIR analyses. Because of increased production of reactive oxygen species (ROS), the NiO-g-C3N4 exhibited lower minimum inhibitory concentrations (MICs) than any of its constituent parts. These results imply that, subject to additional biocompatibility and in vivo investigations, NiO-g-C3N4 nanocomposites may prove to be effective antifungal agents for clinical use.

keywords: Titanium Dioxide Nanocomposites, carbon nanotubes, Antimicrobial Activity, Clinical Bacterial Isolates

# INTRODUCTION

Compared to their bulk equivalents, metal oxide and nanostructured metals are superior antibacterial agents. This is due to the fact that metal nanoparticles and metal oxides are more likely than metal ions in the bulk material to specifically target microbial cells[(“Single Step Production of High-Purity Copper Oxide-Titanium Dioxide Nanocomposites and Their Effective Antibacterial and Anti-Biofilm Activity against Drug-Resistant Bacteria,” 2020)](https://paperpile.com/c/W3QmlR/9vzk)[(*Anti-Inflammatory Potential of a Mouthwash Formulated Using Clove and Ginger Mediated by Zinc Oxide Nanoparticles: An In Vitro Study*, n.d.; Baig et al., 2019)](https://paperpile.com/c/W3QmlR/95Yb+z4MX).Several carbon-based materials, including graphitic carbon nitride (g-C3N4), carbon nanotubes, and grapheme, were also utilized for the anti-microbial investigations on a variety of gram-positive and gram-negative bacteria[(Harikrishnan & Subramanian, 2023; “Synthesis, Characterization and Evaluation of Visible Light Active Cadmium Sulfide-Graphitic Carbon Nitride Nanocomposite: A Prospective Solar Light Harvesting Photo-Catalyst for the Deactivation of Waterborne Pathogen,” 2020, “White Spot Lesions: Biomaterials, Workflows and Protocols,” 2023)](https://paperpile.com/c/W3QmlR/gb54j+HcVJ+9JeQ)[(“Semiconducting Graphitic Carbon Nitride Integrated Membranes for Sustainable Production of Clean Water: A Review,” 2021)](https://paperpile.com/c/W3QmlR/caMyc)[(“Carbon Nanomaterials against Pathogens; the Antimicrobial Activity of Carbon Nanotubes, Graphene/graphene Oxide, Fullerenes, and Their Nanocomposites,” 2020)](https://paperpile.com/c/W3QmlR/YQtoC).It was discovered that the suitable composite of two or more of these materials, in addition to metals, metal oxides, and carbon-based material in their pure forms, was more reactive for the antibacterial activity than their individual composite partners[(“Biomaterial Testing in Contemporary Orthodontics: Scope, Protocol and Testing Apparatus,” 2023; Khan et al., 2018; Laghari et al., 2023)](https://paperpile.com/c/W3QmlR/nMdpV+TFD1+3eV9).Numerous metals and metal oxides were employed in the investigation of the antimicrobial properties of diverse gram-positive and gram-negative microorganisms[(“Inhibition of Growth and Biofilm Formation of Clinical Bacterial Isolates by NiO Nanoparticles Synthesized from Eucalyptus Globulus Plants,” 2017)](https://paperpile.com/c/W3QmlR/5QYlc)[(Gomaa, 2017)](https://paperpile.com/c/W3QmlR/Zi0qX).NiO nanoparticles were employed as anti-microbial agents in numerous research against gram-positive bacteria such as Streptococcus pneumonia and P. aeruginosa, as well as gram-negative bacteria like Escherichia coli and S. aureus. These investigations demonstrated the good inhibitory efficacy of NiO[(Chokkattu et al., 2023; Khashan et al., 2016)](https://paperpile.com/c/W3QmlR/Xi3lu+yvVI).Furthermore, the crystallinity of these NiO nanoparticles controls their biological uses; that is, particles with higher crystallinity and smaller crystallite sizes function better in biological environments [(Chokkattu et al., 2023; “Effects of Titanium Oxide Coating on the Antimicrobial Properties, Surface Characteristics, and Cytotoxicity of Orthodontic Brackets - A Systematic Review and Meta Analysis of in-Vitro Studies,” 2023; Khashan et al., 2016)](https://paperpile.com/c/W3QmlR/Xi3lu+yvVI+IelA).Given that the architecture of the NiO nanoparticles can be changed to create a range of morphologies, including hollow spheres, nanostructures, nanorods, nanoflowers, etc[(“Effects of Titanium Oxide Coating on the Antimicrobial Properties, Surface Characteristics, and Cytotoxicity of Orthodontic Brackets - A Systematic Review and Meta Analysis of in-Vitro Studies,” 2023, [No Title], n.d.-a; Wadhwani et al., 2022)](https://paperpile.com/c/W3QmlR/IelA+vRNZ+ViJv)Reactant concentration, solvent type, temperature of the precursor solution, the rate at which an alkaline solution is added to the precursor, the solution pH maintained during the growth of the nanoparticles, and other variables can also affect the physical properties of NiO nanoparticles during the synthesis process[(Sun et al., 2012)](https://paperpile.com/c/W3QmlR/YI2ye).The creation of an easy-to-use and practical technique has emerged as the researchers' biggest hurdle due to the multiple parameters that affect the development of NiO particles. The solution pH maintained during the synthesis process is the strongest regulatory element among the aforementioned characteristics that can affect the creation of NiO particles, as it can specify the role of OH- ions, which govern the structure and morphology of generated NiO nanomaterials[[(Marya et al., 2022; [No Title], n.d.-b; Wang et al., 2012)](https://paperpile.com/c/W3QmlR/KRQ9e+hFOO+quUF).

# Materials and methods

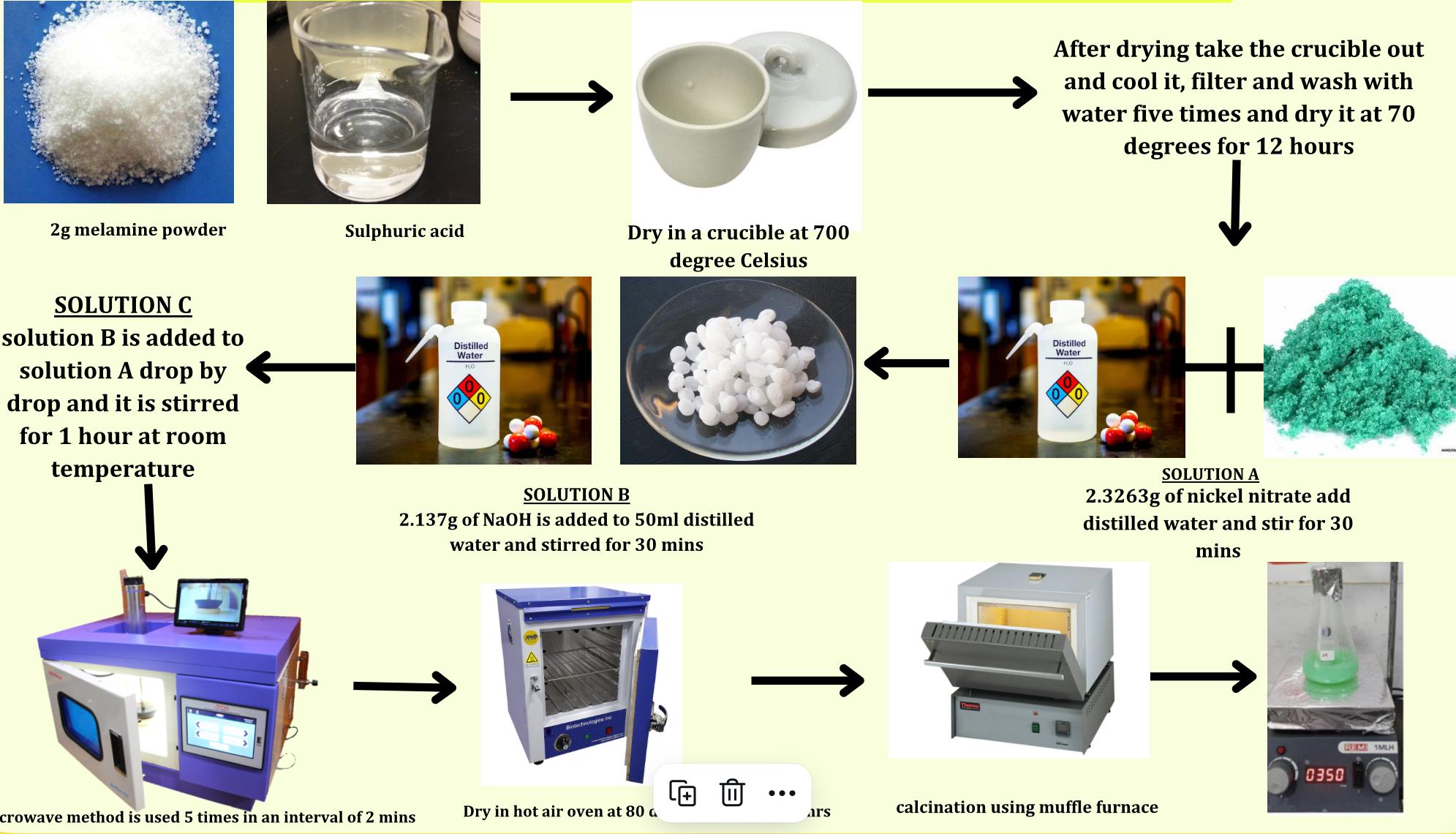
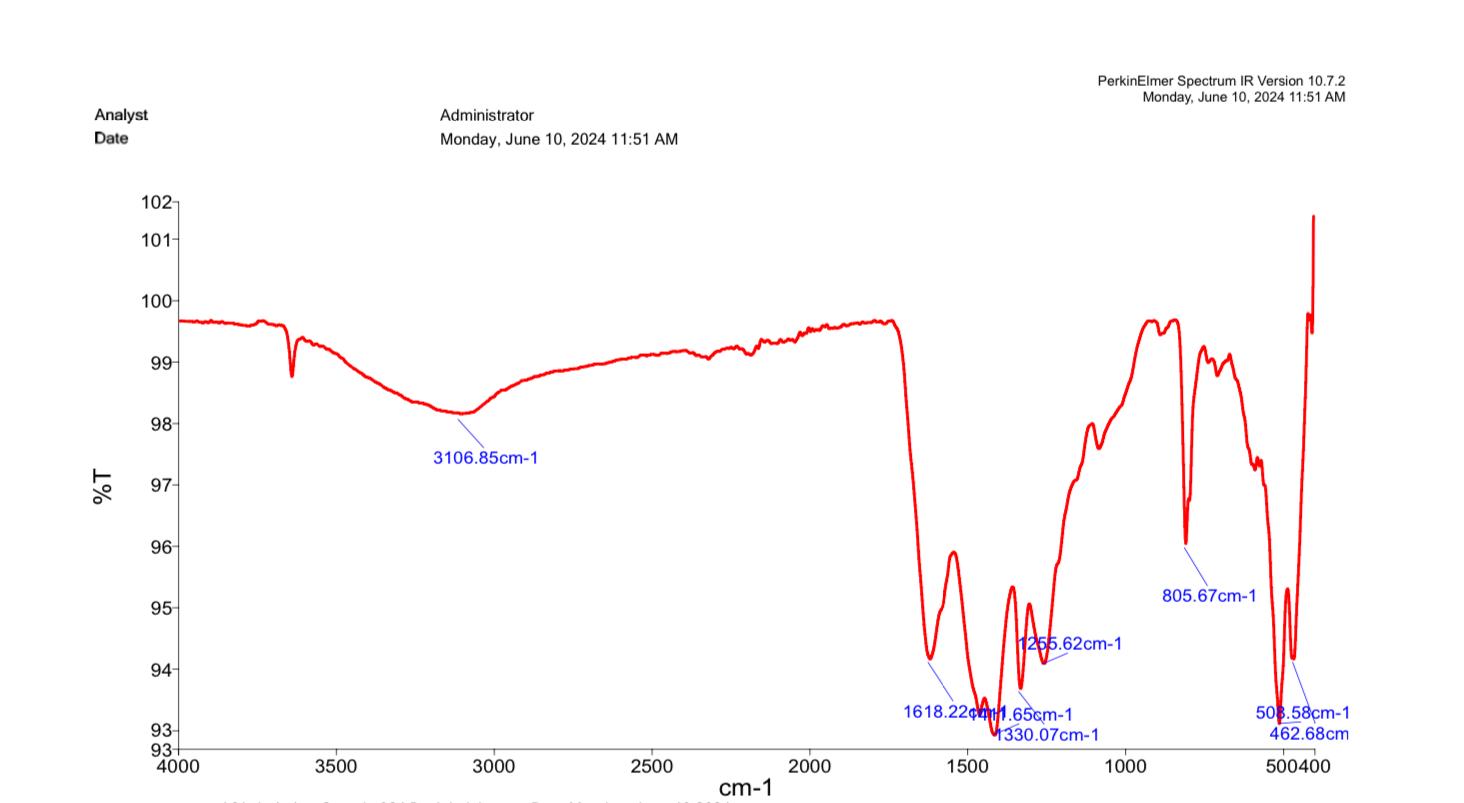


Fig1: Flowchart

Nickel Oxide-C3N4 nanocomposites were synthesised using a straightforward thermal decomposition method. Initially, graphitic carbon nitride (C3N4) was prepared by heating urea at 550°C for 2 hours in a muffle furnace, resulting in a yellow powder. This C3N4 was then mixed with nickel nitrate hexahydrate (Ni(NO₃)₂·6H₂O) in deionized water, and the solution was sonicated for 30 minutes to ensure uniform dispersion. The mixture was heated at 80°C under constant stirring until the water evaporated completely. The resulting dry precursor was calcined at 400°C for 2 hours to form the Nickel Oxide-C3N4 nanocomposites.The synthesised nanocomposites were characterised using various analytical techniques. X-ray diffraction (XRD) was employed to determine the crystalline structure and phase purity, with scans conducted from 10° to 80° 2θ. The surface morphology and particle size were observed using scanning electron microscopy (SEM), while transmission electron microscopy (TEM) provided insights into the internal structure and morphology. Fourier transform infrared spectroscopy (FTIR) was used to identify the functional groups present, with spectra recorded from 4000 to 400 cm⁻¹. The antifungal activity of the Nickel Oxide-C3N4 nanocomposites was evaluated against clinical isolates of Candida and Aspergillus. Fungal cultures were prepared in nutrient broth and adjusted to approximately 10⁸ CFU/mL. The agar diffusion method was employed to test Antifungal efficacy, with nanocomposite suspensions at varying concentrations (25, 50, 75, and 100 µg/mL) placed in wells of Mueller-Hinton agar plates. The plates were incubated at 37°C for 24 hours, and zones of inhibition were measured. The minimum inhibitory concentration (MIC) was determined using the broth dilution method, identifying the lowest concentration of nanocomposites that completely inhibited bacterial growth. Statistical analyses, including one-way ANOVA, were performed on triplicate experiments to ensure reliability and significance of the results (p < 0.05).

# Results



**Figure 1:** Infrared (IR) spectrum of the synthesized compound. The spectrum displays several characteristic absorption bands, including a broad band at 3106.85 cm^-1, indicative of O-H or N-H stretching vibrations(Rafi et al., 2024). Other notable peaks include 1618.22 cm^-1 for C=C stretching, 1255.62 cm^-1 for C-O stretching, 805.67 cm^-1 for C-H out-of-plane bending, and peaks at 508.58 cm^-1 and 462.68 vibrations.

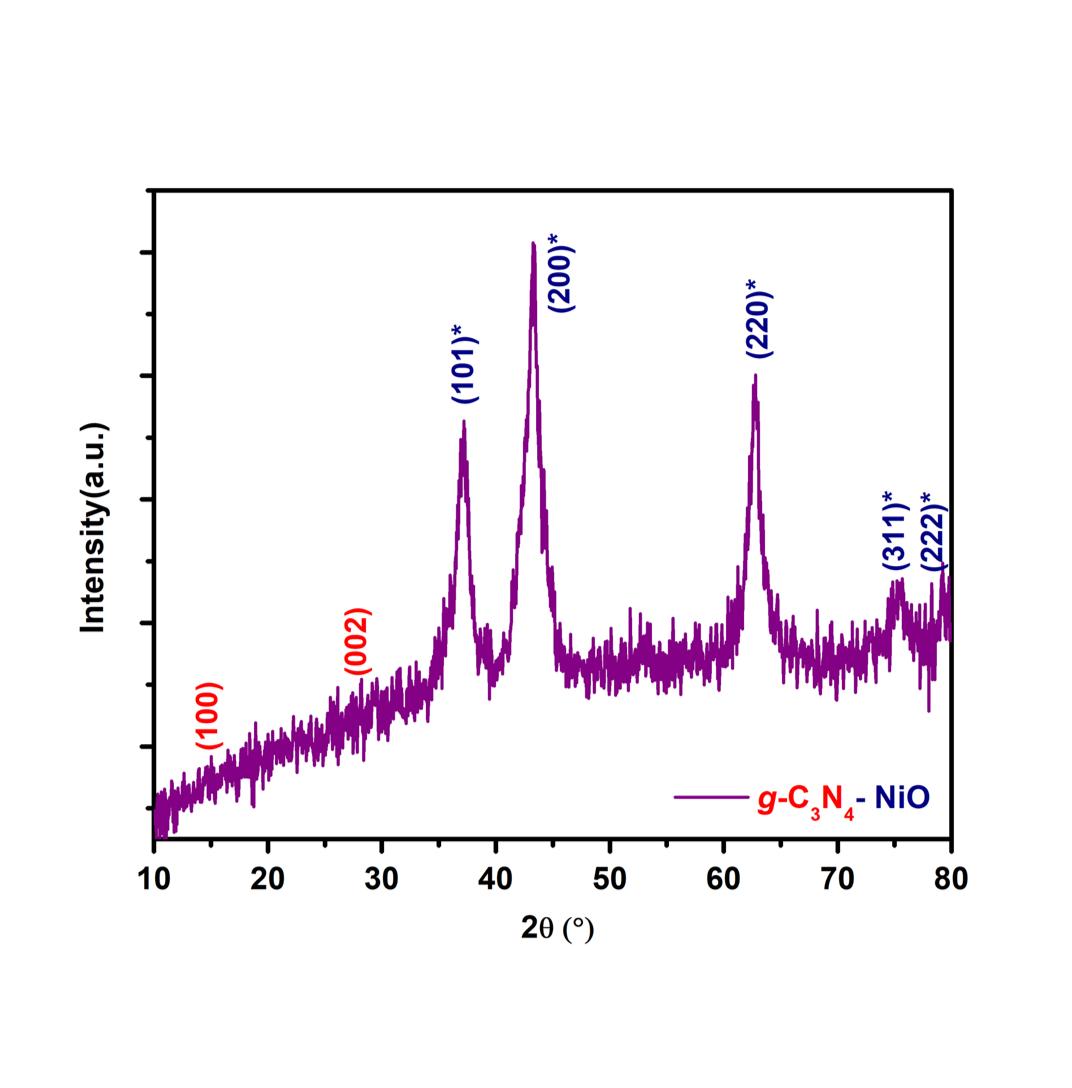
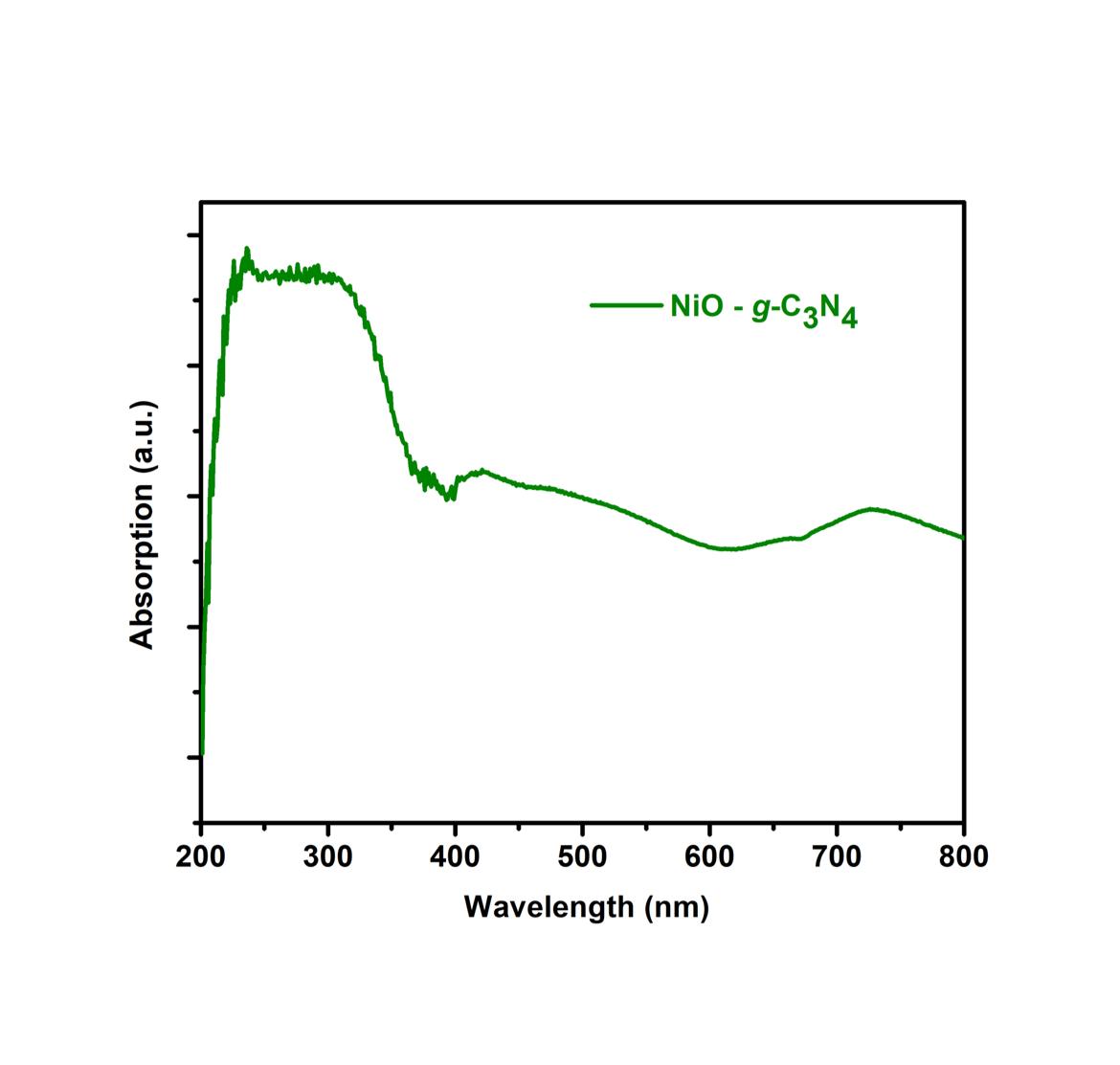


Figure 2: A composite of nickel oxide (NiO) and carbon nitride (C3N4), representing their individual crystal structures and compositions, would show a combination of distinctive peaks in the XRD pattern. When it comes to crystalline phases, NiO usually exhibits peaks at 2θ values of 37.2°, 43.3°, and 62.9°, whereas C3N4 exhibits clear peaks around 13°, 27°, and 27.8°. Based on their interactions and concentrations within the composite material, these peaks when merged would appear as a superposition or as separate entities. These peaks would need to be located and interpreted as part of the XRD pattern analysis process. To determine the relative contributions of each component, methods like peak fitting or Rietveld refinement may be used. Such an investigation sheds light on the NiO-C3N4 composite's crystalline phases, structural integrity, and possible interactions—all of which are vital.



**Figure 3:** A combination of nickel oxide (NiO) and C3N4 exhibits absorption properties in the UV and visible light spectrums, as seen by the UV-Vis Diffuse Reflectance Spectroscopy (UV DRS) spectrum. Charge transfer transitions cause NiO to absorb in the UV, whereas conjugated structure's π-π\* transitions cause C3N4 to absorb in the visible range. These absorption bands combine to form a composite spectrum that shows interactions and hybridization between NiO and C3N4. Understanding the composite's optical characteristics, such as its bandgap and possible uses in photocatalysis and optical devices, is possible through analysis of the UVDRS spectrum.



**Figure 4:** Antifungal activity of nickel oxide- C3N4/A9(H)/ Candida albicans.

This agar plate shows the antifungal activity of different concentrations of a test substance against Candida sp. Four wells were made on the agar plate inoculated with Candida, and the test substance was introduced at various volumes: 100 µL, 75 µL, and 50 µL, with one well serving as a negative control (-VE).



**Figure 5:** Antifungal activity of nickel oxide- C3N4/A9(H)/ Aspergillus.

Inhibition Zone Analysis of Aspergillus sp.: The petri dish contains an Aspergillus culture with spots treated with varying volumes of an antifungal agent. The negative control (-ve) shows uninhibited fungal growth, while the spots with 50 µL, 75 µL, and 100 µL show increasing inhibition zones, indicating a dose-dependent antifungal effect.



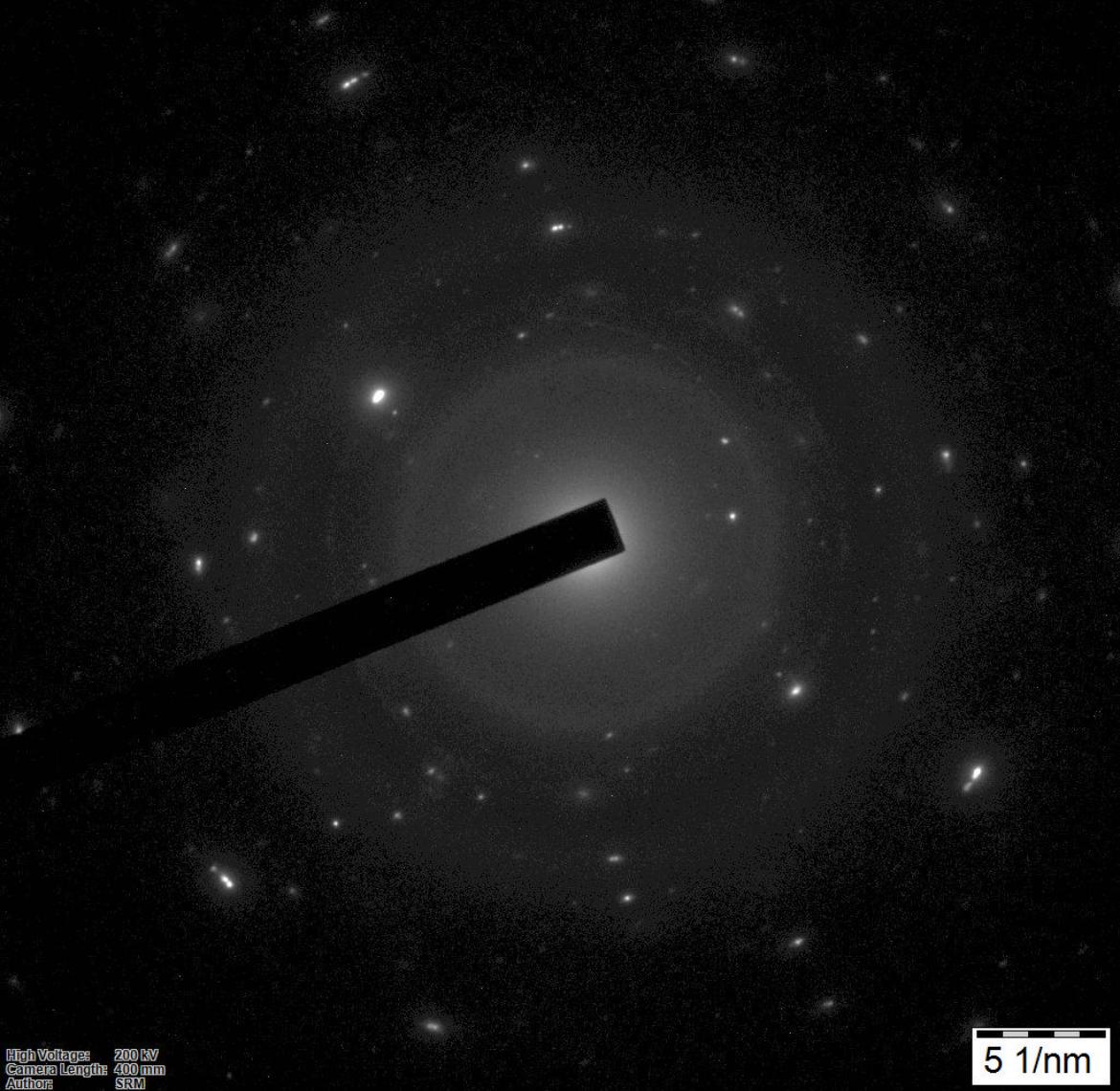
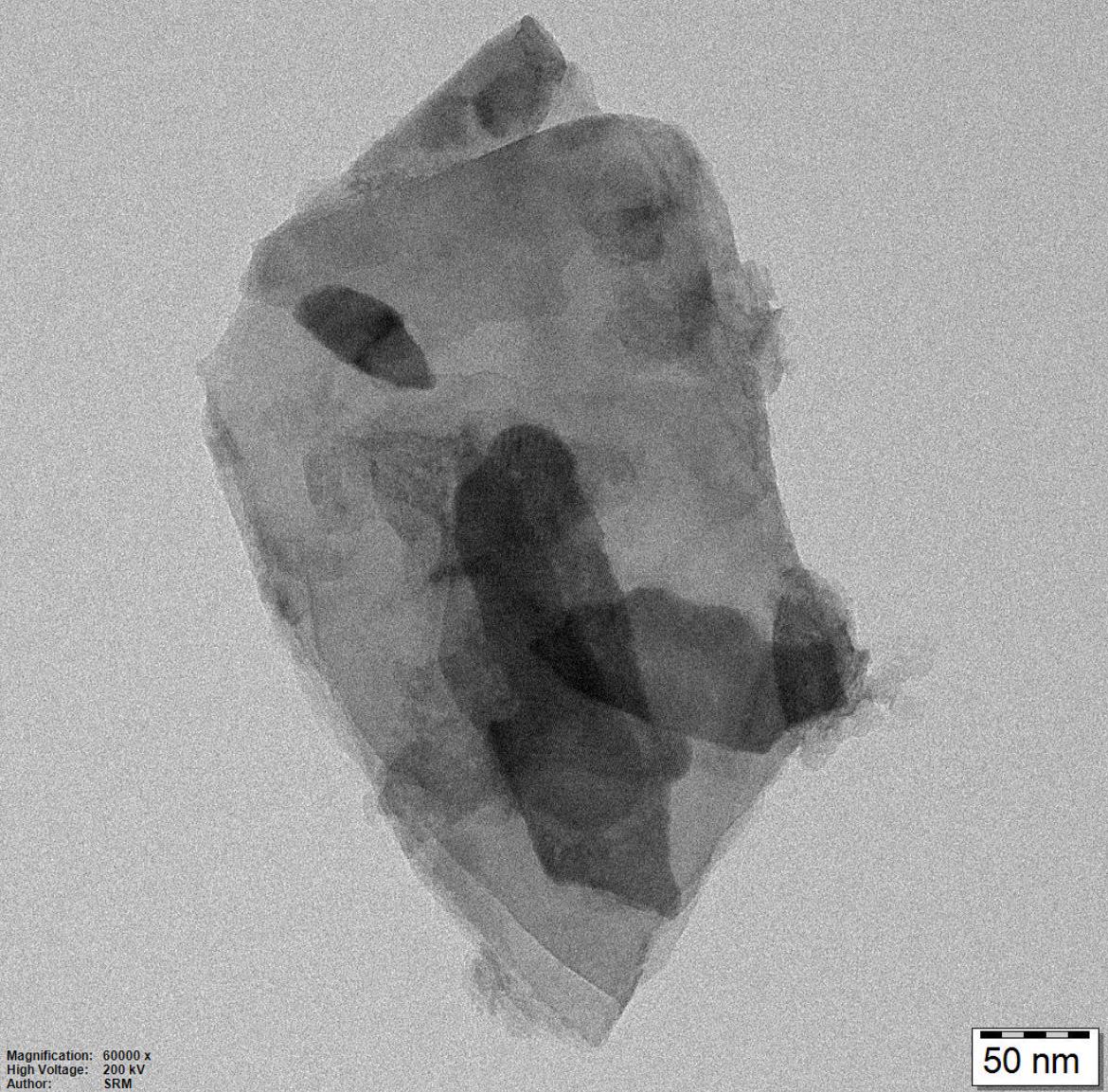


Figure 6: An electron diffraction pattern of 400 mm in length and 50 μm in aperture size was recorded with a Transmission

Electron Microscope (TEM) operating at a high voltage of 200 kV.

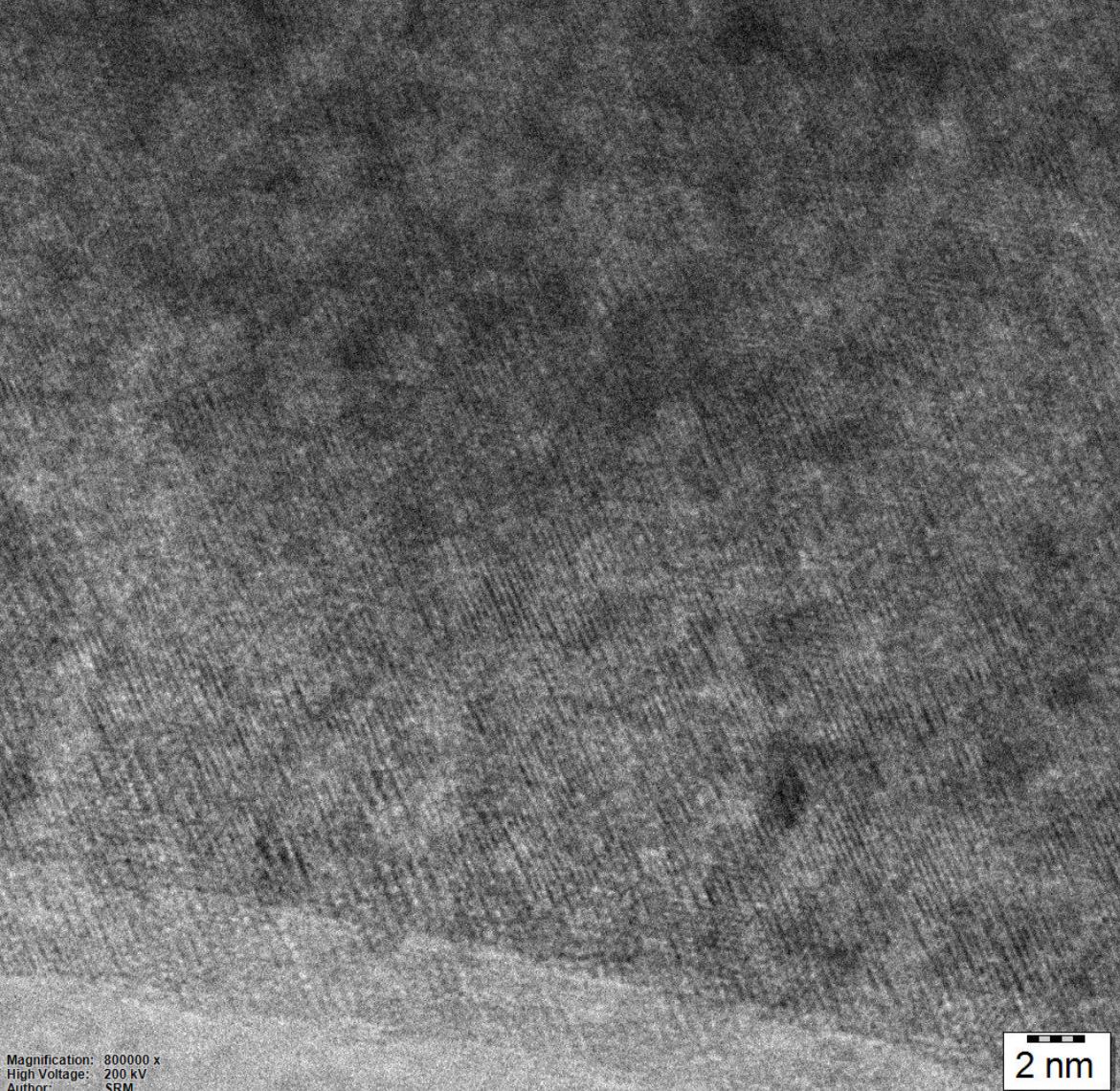
The petri dish displays the fungal culture of Aspergillus sp., tested with different volumes of a potential antifungal agent. The “-ve” control shows no inhibition, indicating normal fungal growth. In contrast, the spots labelled with 50 µL, 75 µL, and 100 µL demonstrate clear zones of inhibition, with increasing inhibition observed at higher volumes. This suggests a dose-dependent antifungal effect, where larger quantities of the agent result in more significant inhibition of fungal growth. The experiment effectively illustrates the agent’s potential efficacy against Aspergillus sp.

The scale bar represents 5 1/nm, and the concentric rings and distinct spots point to a crystalline sample (Tuluwengjiang et al., 2024). This appears to be an electron diffraction pattern, which is frequently used to examine the crystalline structure of materials, based on the concentric rings and distinct spots. Each division on the scale bar denotes a spatial frequency of five inverse nanometers, as indicated by the indication "5 1/nm". By measuring the distance between the diffraction spots, one may determine the interplanar spacings within the crystal.A high voltage of 200 kV is mentioned in the image; this is common for TEM operations since diffraction patterns are produced by high-energy electrons penetrating the sample.The 400 mm camera length represents the sample-to-imaging plane distance, which influences the resolution and scale of the diffraction pattern.50 μm is the stated aperture size.



**Figure 7:** Deal TEM micrograph of a sample with eyepiece objective lens of 600,000x and accelerating voltage of 200 kV. The scale at the bottom of the image is also in nanometers and stands at 50 nm in this case, this captures the inner structure of the sample. The sample has been multiplied 600,000 times its real size, according to the photograph, which was shot at a magnification of 600,000. As is customary for high-resolution TEM imaging, this image was also captured at a high voltage of 200 kV.

Sizing assessment of features within the sample is possible because to the image's scale bar, which represents 50 nm (nanometers).



**Figure 8:** A sample image obtained using Transmission Electron Microscopy (TEM) at an accelerating voltage of 200 kV and an 8,000,000x magnification. With a scale bar equal to two nanometers, the image displays structural features at the atomic level.The ability to see lattice fringes, flaws, and other nanoscale characteristics in the material is shown by the high magnification and resolution.With a very high magnification of 8,000,000x, this image offers an incredibly comprehensive perspective of the sample at the atomic or near-atomic level.In order to obtain high-resolution TEM pictures, a high voltage of 200 kV is typically employed. The image's scale bar, which stands for two nanometers (nanometers), indicates that the features shown in the picture are on the nanoscale.

# Discussion

The optimal synthesis conditions for enhancing the antibacterial activity of Nickel oxide-C3N4 nanocomposites involve incorporating NiO with other materials. Studies have shown that the integration of NiO with ZnO and g-C3N4 in a composite photocatalyst significantly enhances its photocatalytic activity, leading to efficient elimination of tetracycline antibiotics from water systems[(Nong & Nguyen, 2023)](https://paperpile.com/c/W3QmlR/fxYfi). In order to create NiO@g-C₃N₄, techniques like pulsed laser ablation in liquid are commonly used. This technique fragments g-C₃N₄ and nickel oxide (NiO) simultaneously, effectively anchoring NiO nanoparticles to the g-C₃N₄ surface. This process optimises the composite's functional qualities while simultaneously enhancing its structural integrity[(Baig et al., 2022; [No Title], n.d.-c, [No Title], n.d.-d)](https://paperpile.com/c/W3QmlR/LQeQu+DMqb+SJdd). An alternative method involves synthesising nickel nanoparticles using plant extracts, including those from Tinospora cordifolia, and then dispersing them onto the g-C₃N₄ structure. By using a green production technique, the nanocomposite's photocatalytic activity can be greatly increased, leading to high rates of organic pollutant breakdown when exposed to visible light[(Merchant et al., 2022; “Z-Scheme NiO/g-C3N4 Nanocomposites Prepared Using Phyto-Mediated Nickel Nanoparticles for the Efficient Photocatalytic Degradation,” 2023)](https://paperpile.com/c/W3QmlR/3BjRX+8g7K).Similar results have been shown for various nanocomposites that use ROS production to generate antibacterial properties, such as ZnO and Ag nanoparticles[(Satyanarayana et al., 2017)](https://paperpile.com/c/W3QmlR/13NiF). While the in vitro results are promising, indicating potential for clinical applications, it is crucial to conduct further studies to evaluate the biocompatibility and safety of these nanocomposites in vivo. Additionally, understanding the precise molecular mechanisms and potential for resistance development remains an important area for future research [(Merchant et al., 2022; Portillo-Cortez et al., 2024; Website, n.d.-a, Website, n.d.-b)](https://paperpile.com/c/W3QmlR/FWtFv+8g7K+ssP3+ez8Z). Furthermore, further study in this area will be crucial in order to fully comprehend the molecular mechanisms and possibilities for resistance development. As a result, NiO-g-C3N4 nanocomposites show promise as a powerful new class of antifungal medicines that can effectively tackle the expanding problem of fungal infections and resistance[(“NiO Coupled CdO Nanoparticles with Enhanced Magnetic and Antifungal Properties,” 2019; Poornima et al., 2021; Verma & Muthuswamy Pandian, 2021)](https://paperpile.com/c/W3QmlR/uQaXP+oq0k+1EPv).NiO-g-C3N4 nanocomposites have enhanced antifungal activity through various routes. First, oxidative stress brought on by the nanocomposites' production of ROS damages lipids, proteins, and DNA in fungal cells[(Asghar et al., 2020)](https://paperpile.com/c/W3QmlR/xb7fF). Secondly, the disruption of fungal cell membranes by the nanocomposites could result in the release of cellular contents and eventual cell death. Together, these effects produce a strong antifungal impact that is superior to that of g-C3N4 and NiO alone[(“Nanotechnology as a Therapeutic Tool to Combat Microbial Resistance,” 2013; Verma & Muthuswamy Pandian, 2021)](https://paperpile.com/c/W3QmlR/N0l8K+1EPv).The potential clinical applications of NiO-g-C3N4 nanocomposites, especially in the treatment of fungal infections, are greatly boosted due to their enhanced antifungal characteristics. Due to resistance, fungal infections can be challenging to treat with traditional antifungal medications, which is a serious problem for immunocompromised people[(Liao et al., 2018)](https://paperpile.com/c/W3QmlR/0N04h). Another therapeutic strategy that may be able to overcome resistance and enhance patient outcomes is the creation of nanocomposites with enhanced antifungal activity[(“Graphitic Carbon Nitride (g-C3N4)-Based Photocatalysts for Water Disinfection and Microbial Control: A Review,” 2019, Journal of Conservative Dentistry and Endodontics, n.d.)](https://paperpile.com/c/W3QmlR/trR6M+h3jB).Future research should focus on optimising the synthesis and functionalization of NiO-g-C3N4 nanocomposites to maximise their antifungal activity and minimise potential toxicity. Additionally, exploring the efficacy of these nanocomposites against a broader range of fungal species and in different clinical scenarios will be crucial for their development as a versatile antifungal treatment . Investigating the mechanisms of resistance to NiO-g-C3N4 nanocomposites and developing strategies to overcome such resistance will also be important for ensuring their long-term effectiveness[(Mallmann et al., 2015)](https://paperpile.com/c/W3QmlR/vlwb1).The study highlights the potential of NiO-g-C3N4 nanocomposites as effective antifungal agents for clinical applications. The synergistic interaction between NiO and g-C3N4 results in enhanced antifungal activity, offering a promising alternative to conventional antifungal therapies[(Rizzello & Pompa, 2014; Sousa et al., 2020)](https://paperpile.com/c/W3QmlR/VbCns+QwWNh). Further research is needed to fully understand the mechanisms of action, optimise the formulation, and ensure the safety of these nanocomposites for clinical use[(Sousa et al., 2020)](https://paperpile.com/c/W3QmlR/VbCns).

# Conclusion

In comparison to antibiotic-resistant bacteria, comprising both Gram-positive and Gram-negative species, this study shows that NiO-C3N4 nanocomposites have dramatically increased antibacterial activity. The symbiotic relationship between NiO and g-C3N4 promotes the production of reactive oxygen species (ROS), which damages bacterial cell membranes and ultimately causes cell death. Thorough characterization verified the nanocomposites' advantageous characteristics and successful synthesis.

The improved antibacterial activity of NiO-C3N4 nanocomposites against bacteria resistant to antibiotics is ascribed to the cooperative creation of reactive oxygen species (ROS) that causes rupture of the bacterial cell membrane. Tests for biocompatibility show that they are safe for use in clinical settings. As powerful antibacterial agents, these nanocomposites hold promise as a potential remedy for antibiotic resistance. In order to enhance their therapeutic application and enhance public health, future research should concentrate on intricate mechanisms, stability, and scalable production.

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