Investigating the Antimicrobial Mechanisms of Molybdenum Carbide-Nickel Sulphide Composites

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**Abstract:** Antimicrobial resistance is becoming a growing public health concern ,specifically with the increase in emergence of antibacterial -resistant bacteria. This study investigates the potential of molybdenum carbide-nickel sulfide composites to be used to combat these superbugs. This novel approach involves the creation of composites through the combination of molybdenum carbide and nickel sulfide nanoparticles. Advanced techniques such as Transmission electron microscopy (TEM), High-resolution TEM (HR-TEM), X-ray diffraction (XRD), UV-visible diffuse reflectance spectroscopy and Fourier-transform infrared technique are applied for the structural analysis of these composites. The antimicrobial efficiency of the composites is assessed through bactericidal activity assays against a variety of bacterial strains and fungi, including Aspergillus and Candida albicans. Reduced particle size and increased surface area improve contact with microbial cells, enhancing the efficacy of antimicrobial drugs. Mechanistic experiments will be conducted to explain the antibacterial activity by looking into the composites' interactions with bacterial membranes, the potential formation of reactive oxygen species (ROS), and the release of metal ions. The goal of our research is to develop these composites as powerful instruments against antibiotic resistance, which could rapidly enhance public health outcomes.

**Keywords**: biocompatibility, antimicrobial mechanism, sulfide nanoparticles, Centrifugation, Fourier Transform Infrared Spectroscopy

# Introduction

Globally, infectious diseases have a significant adverse impact on the economy and society[(“Emergent Crisis of Antibiotic Resistance: A Silent Pandemic Threat to 21st Century,” 2023)](https://paperpile.com/c/VybVEW/hMRRO). Antibiotic resistance is one of the main issues facing world healthcare in the twenty-first century. There have been reports of resistance to traditional antibiotics in several drugs used in clinical settings. Antibiotic resistance could be viewed as the "Faceless Pandemic" that is causing uneasiness throughout the world. The primary causes for antibiotic resistance are the misuse and abuse of antibiotics, a lack of innovative drugs in research, the profit-driven mentality of the healthcare sector, and the absence of diagnostic testing before antibiotic prescribing. The public is now in danger of a variety of health issues as a result of the overuse of antibiotics, including superbugs that are immune to all known therapies [(Khameneh et al., 2016)](https://paperpile.com/c/VybVEW/PRWfp). Major public health concerns remain unresolved antibiotic resistance and incurable bacterial diseases. Therefore, an environmentally friendly approach with strong antibacterial efficacy against bacteria and biocompatibility is desperately needed. Clinical investigations have shown that the lack of anti-caries capabilities in dental filling and bonding materials over a long period resulted in a high prevalence of secondary caries. In caries prevention and treatment, the development of novel anti-caries materials is a prevalent topic. The effectiveness of dental caries prevention techniques has greatly improved in recent decades. The use of nanoparticles (NPs) in the creation of anti-caries agents has drawn a lot of interest [(Harsha & Subramanian, 2022)](https://paperpile.com/c/VybVEW/ftDPK). Given the current issues and difficulties in caries prevention and treatment, in addition to the special benefits of nanomaterials, anti-caries nanomaterials have emerged as a significant advancement in caries research [(Al-Hijazi et al., 2023)](https://paperpile.com/c/VybVEW/5FKl6). As a substitute approach to combating harmful germs, nanotechnology has shown remarkable bactericidal efficacy, minimal susceptibility to resistance emergence, and sufficient biosafety.Nanoparticles are materials with at least one dimension between 1 and 100 nanometers, or whose fundamental unit in three dimensions falls within this range [(Chidambaram et al., 2022)](https://paperpile.com/c/VybVEW/X5Qu4). Nanoparticles are currently widely used in a variety of technologies, including medication, cosmetics, agriculture, and food sciences. Nanoparticles in particular have shown a broad spectrum of antibacterial properties, against both Gram-positive and Gram-negative bacteria [(Tiwari & Jain, 2023)](https://paperpile.com/c/VybVEW/taFt1)[(Ramalingam et al., 2016)](https://paperpile.com/c/VybVEW/HLcUJ). NPs' antimicrobial mechanism of action is commonly explained by their association with one of three models: non-oxidative mechanisms [(Gurunathan et al., 2012)](https://paperpile.com/c/VybVEW/XpQ8i), metal ion release[(Nagy et al., 2011)](https://paperpile.com/c/VybVEW/EymgU), or oxidative stress induction [(Govindaraj & Dinesh, 2021)](https://paperpile.com/c/VybVEW/F3Xtg)[(Leung et al., 2014; Wang et al., 2017)](https://paperpile.com/c/VybVEW/I54KK+VdZLY). Additionally, the production of reactive oxygen species (ROS) damages the cell membrane mechanically and impairs the antioxidant defense system. Recent studies have revealed that the following main mechanisms underlie NPs' antimicrobial effects: The bacterial cell membrane can be disrupted, reactive oxygen species (ROS) can be produced, the bacterial cell membrane can be penetrated, and intracellular antibacterial effects, including interactions with DNA and proteins, can be induced [(Balaji Ganesh S & Sugumar, 2021)](https://paperpile.com/c/VybVEW/h2Z8i) [(Shaikh et al., 2019)](https://paperpile.com/c/VybVEW/0bdxs). The intravenous administration of drugs encapsulated in the nanocarriers promotes their localization in specific tissues as a consequence of the anatomical functions and physiological characteristics of the various body compartments [(Jabin et al., 2021)](https://paperpile.com/c/VybVEW/LXZx4). Inorganic NPs have been shown to suppress dental caries by reducing biofilm development and remineralizing carious lesions in recent research studies [(Hannig & Hannig, 2010)](https://paperpile.com/c/VybVEW/t1s7E). Biomineralization is stimulated by metallic NPs *via* facilitating remineralization of demineralized (carious) tooth tissues. Furthermore, due to their ion balance in oral fluid, metallic NPs can overcome problems in a variety of oral disorders [(Katyal et al., 2021)](https://paperpile.com/c/VybVEW/AeUpl)[(Nizami et al., 2021)](https://paperpile.com/c/VybVEW/qC2E7).To prevent caries development and strengthen the mechanical properties of the materials, the metal and metal oxide NPs have been incorporated into dental materials. In addition, inorganic nanomaterials can be used as direct antibacterial agents, and then inhibit dental caries, because it is one of the most common bacteria-related oral diseases [(Zhu et al., 2022)](https://paperpile.com/c/VybVEW/clbLy). Inorganic nanoparticles are expected to have potential applications in elevating the quality of human life and improving mankind’s medical conditions by employing early detection, diagnosis, treatment and follow-up of various diseases [(Ajay, Suma, et al., 2022)](https://paperpile.com/c/VybVEW/zEbr1).Because of its ability to improve drug absorption, precise delivery, and minimize side effects, nanotechnology offers a solution to persistent infectious disease challenges posed by multidrug-resistant organisms [(Ajay, Sasikala, et al., 2022)](https://paperpile.com/c/VybVEW/5gB2K).. While organic nanoparticles (NPs) have the potential to improve antibiotic efficacy due to their biodegradability and biocompatibility, nanoparticles (NPs) can target multiple components of bacteria and combat multidrug resistance through multi-component bacterial targeting [(Khorsandi et al., 2021)](https://paperpile.com/c/VybVEW/eAjjh). Recent findings highlight the potential of combining antibiotics with metallic nanoparticles to sustain bacterial sensitivity to these drugs, offering a promising avenue. To improve biocompatibility, drug-loading, and sustained drug release, non-toxic polymers are either used in the form of nanoparticles or coated on inorganic nanoparticles like Nickel-containing nanoparticles (nickel oxide NPs) which have been shown to possess biocompatibility and chemical stability. The undoped nickel sulfide nanoparticles showed significant antibacterial activity [(Solanki et al., 2022)](https://paperpile.com/c/VybVEW/4Ovgm). Since nickel sulfide can also exhibit some metallic properties depending on its specific composition and structure and is also a conductor of electricity we can consider them to have properties similar to metallic nanoparticles. Various metallic nanoparticles offer distinct advantages in treating bacterial infections, potentially enhancing the effectiveness of commonly employed antibiotics, even against multidrug-resistant microorganisms[(Agreles et al., 2022)](https://paperpile.com/c/VybVEW/mmQWL). Metallic nanoparticles (NPs) have antimicrobial qualities that make them viable substitutes for antibiotics and potent antibacterial agents. Metallic nanoparticle-based platforms exhibit promise as a stand-alone or in conjunction with antibacterial drugs, potentially providing an effective means of addressing antibiotic resistance.Due to its high chemical stability and platinum-like catalytic properties, molybdenum carbide (Mo2C) has recently attracted a lot of attention. It has potential for various fields such as Catalysts, electrochemical sensors, lithium-sulfur batteries, nitrogen reduction and hydrogen evolution reactions. Molybdenum carbide falls under the category of ceramic materials which makes them wear resistant. Researchers are developing ceramic nanomaterials for dental use because ceramic nanoparticles are more stable and cheaper in production than metallic nanoparticles. Ceramic nanoparticles can be utilized to prevent dental cavities because some have mineralising capabilities that encourage tooth tissue remineralization. Ceramic minerals aid in the remineralisation process and keep pH levels stable to maintain tooth integrity. In addition, Ceramic materials are biocompatible, have a high affinity to tooth structure and also exhibit antimicrobial properties against cariogenic microbes [(Graf et al., 2023)](https://paperpile.com/c/VybVEW/GZTu)[(Seyedmajidi et al., 2018)](https://paperpile.com/c/VybVEW/pLpKA). Researchers have developed antimicrobial nanoparticles, conjugated ceramic minerals with antibacterial and mineralising properties, to prevent the formation and progression of caries [(Nizami et al., 2022)](https://paperpile.com/c/VybVEW/W3RNb).

# Materials and method

Molybdenum carbide is synthesized by first weighing 2.0 g of sodium alginate powder and adding it to a beaker. Distilled water (50 ml) is then added, and the mixture is stirred for 30 minutes until colorless and transparent. Ammonium molybdate (1.0 g) is weighed and added to a separate beaker with 50 ml of distilled water. This solution is stirred for 30 minutes. The sodium alginate and ammonium molybdate solutions are then combined in a beaker and stirred for 2 hours. This mixture is subsequently frozen to allow gel formation and lyophilization. The solid gel is then heated at 900 °C for 5 minutes, maintained at that temperature for 6 hours, and then allowed to cool to room temperature. The cooled solid is filtered with distilled water several times using Whatman filter paper to remove contaminants and residual reactants, resulting in a black solid precipitate. This black precipitate is then transferred on the filter paper to a vacuum oven and dried at 80 °C for 12 hours The synthesis of nickel sulfide precipitate follows a similar procedure. Nickel nitrate (2.3263 g) is weighed and transferred to a beaker with 50 ml of distilled water. This solution is stirred for 30 minutes using a magnetic stirrer until it becomes light teal coloured. In a separate beaker, nickel disulfide (2.3776 g) is weighed and then added with 50 ml of distilled water. This solution is stirred for 30 minutes with a magnetic stirrer until it turns yellow. The nickel disulphide solution is then added dropwise to the nickel nitrate solution using a dropper, causing the latter to turn black. Finally, the solution is allowed to sit for 1 hour while stirring continuously.

For the molybdenum carbide and nickel sulfide composite, 1.0 g of molybdenum carbide is added to 25 ml of distilled water in a beaker and stirred for 20 minutes. In another beaker, 0.1 g of carbon is added to 25 ml of distilled water and stirred for 20 minutes. These solutions are then combined. The nickel sulfide solution is subsequently added dropwise to the combined solution while stirring for 3 hours, resulting in a black solution. This solution is microwaved at high temperature for 5 minutes in 2-minute intervals. After microwaving, the solution is cooled to room temperature. The solution is then centrifuged in a vial and transferred to a centrifuge tube. Centrifugation is performed three times with distilled water, followed by two times each with ethanol and acetone to remove impurities. The precipitates are then dried in a hot air oven at 80 °C for 24 hours. Calcination is then performed at 300 °C. The precipitate is heated in a muffle furnace for 3 hours after reaching 300 °C and then allowed to cool to room temperature. Finally, the cooled precipitate is ground using a mortar and pestle and packed for further use. The resulting material is a molybdenum carbide-nickel sulfide composite.

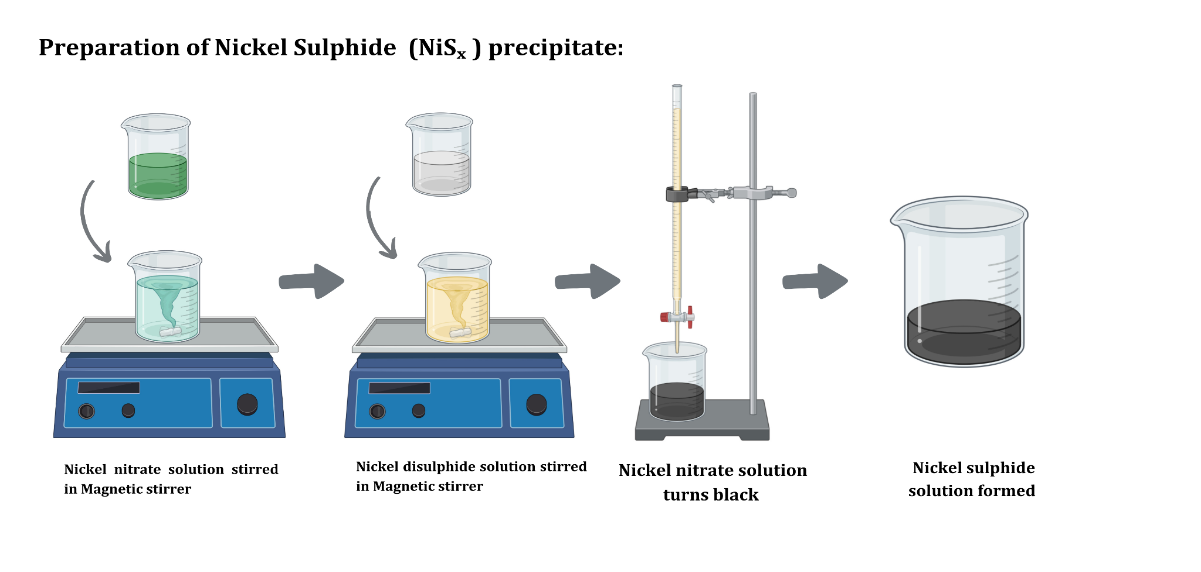


Fig 1: Preparation

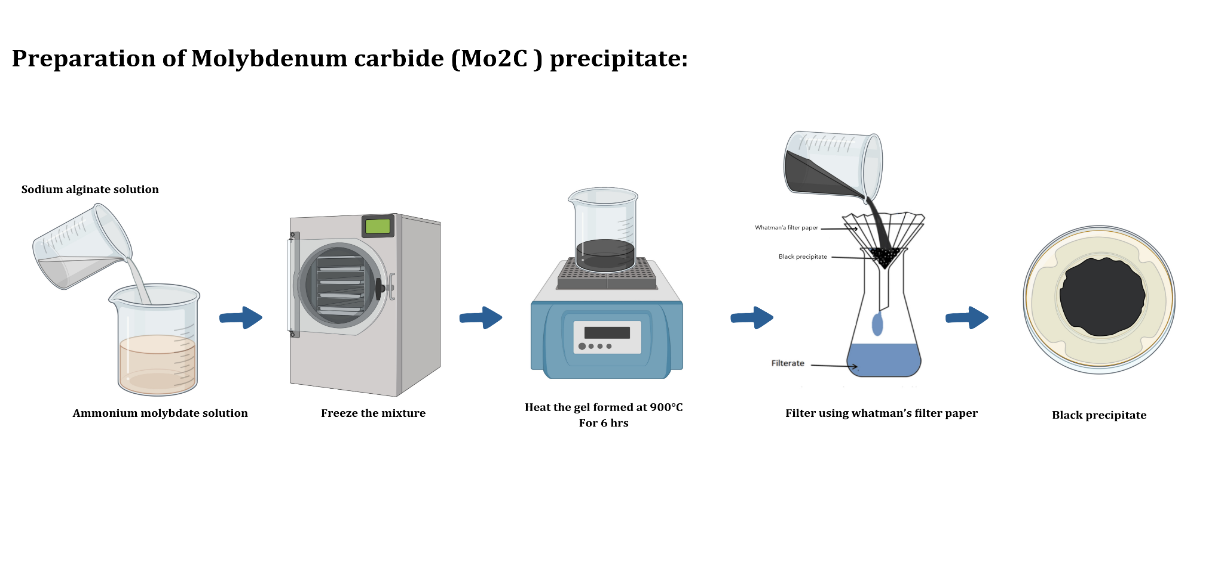


Fig 2: Preparation

# Result

## In graph 1 - UV viz DRS ANALYSIS

Ultraviolet-visible Diffuse Reflectance Spectroscopy (UV-DRS) was used to investigate the optical characteristics and bandgaps of Mo2C-NiS composites.Here the x-axis denotes the wavelength (nm) and y-axis denotes absorption (a.u.).The absorption intensity (a.u.) continuously increases as the wavelength decreases from approximately 800 nm to 200 nm. This means the substance absorbs more light in both ultraviolet (UV) and shows significant absorption light both in ultraviolet and visible regions. This shows that the composite can produce ROS when exposed to light[28,29][(“Tunable Localized Surface Plasmon Resonances in MoO3−x-TiO2 Nanocomposites with Enhanced Catalytic Activity for CO2 Photoreduction under Visible Light,” 2020)](https://paperpile.com/c/VybVEW/gxdkY)[(Addawiyah & Gunlazuardi, 2018)](https://paperpile.com/c/VybVEW/aBu8O).

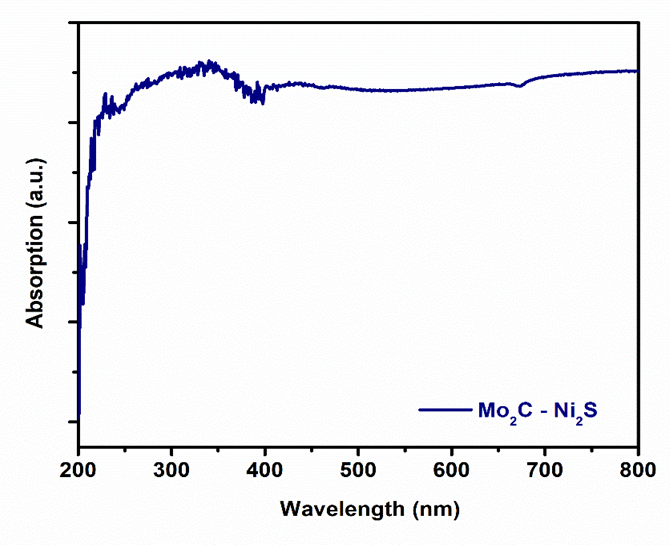


Figure 3: Absorption vs. wavelength

## In graph 2 -XRD Analysis

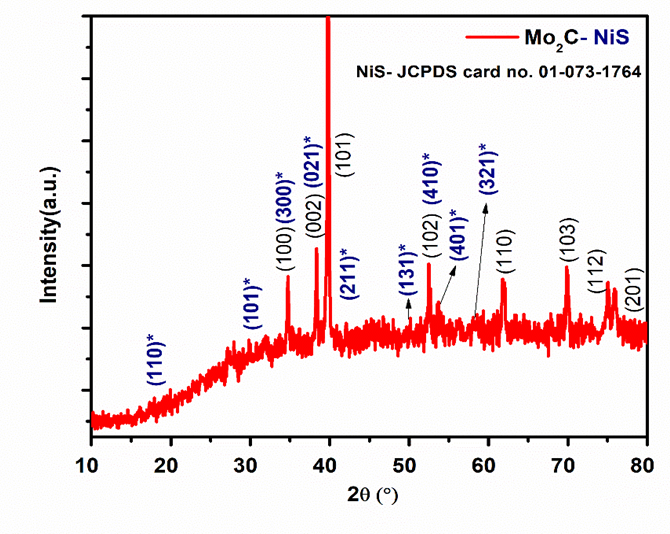


Figure 4: Intensity

In X-Ray diffraction analysis (XRD) the x-axis showed the the angle of detector position from the incident X-ray beam ( 2θ °) and y-axis showed the intensity (a.u.) .Where the XRD Analysis revealed the presence of crystalline composites consisting of Mo2C-NiS composites. The XRD pattern of the molybdenum carbide-nickel sulfide composite (graph 2) exhibits several prominent peaks at 2θ values of 34.4°, 38° and 39.4° These peaks can be assigned to the (100), (002), and (101) reflections of molybdenum carbide (JCPDS #35-0787), respectively. Additionally, a peak at 58.3° corresponds to the (200) reflection of nickel sulfide (JCPDS #87-0719). This confirms the presence of both molybdenum carbide and nickel sulfide phases in the composite. The absence of impurity peaks suggests the successful synthesis of the desired composite. Sharp crystallinity peaks observed are advantageous for catalysis and for antimicrobial mechanism .The occurrence of those same peaks with a little variance in the wavenumber has previously been described [30,31][(Burueva et al., 2019; Ma et al., 2015)](https://paperpile.com/c/VybVEW/xSrjo+idzOu).

## In graph 3 -FTIR Analysis

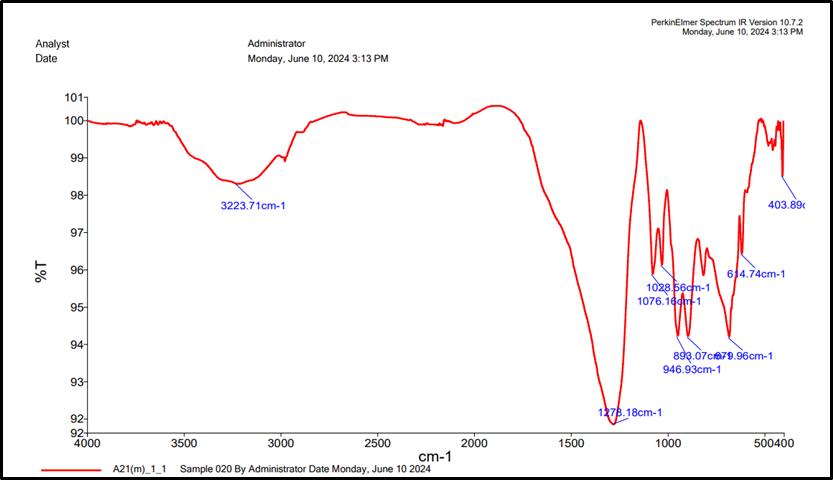
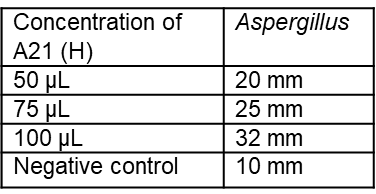


Fig 5: FTIR Analysis

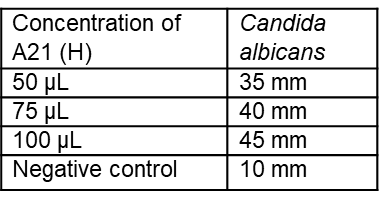
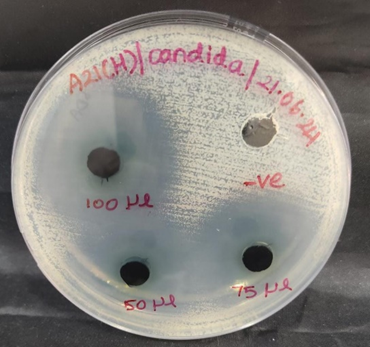
Fourier Transform Infrared Spectroscopy (FTIR) analysis where the x-axis denotes the frequency of wavelength and y-axis denotes the infrared spectrum.The FTIR analysis was performed to identify the functional groups present in the molybdenum carbide-nickel sulfide composite. In Fourier Transform Infrared Spectroscopy (FTIR) the functional groups and chemical bonds of Mo2C -NiS composites were identified .The FTIR peaks that are present in MO2C and NiS were observed in peak 1278.184 corresponding to C=O [ carbonyl group ]. The presence of a composite was confirmed by analyzing the spectra of the Mo-C and Ni-S bonds.From graph 3 , results demonstrated that the Mo2C-NiS composites showed characteristic FTIR peaks (1278.184 for alkyl ketone ) ( 1028-1076 for alkyl amine ).The occurrence of those same peaks with a little variance in wavelength has previously been described[32,33] [(Karadaghi et al., 2022)](https://paperpile.com/c/VybVEW/EGfk8)[(Karthikeyan et al., 2015)](https://paperpile.com/c/VybVEW/QV4UG).

## Antimicrobial assay

The antimicrobial effects may be caused by the interaction between hydrophilicity and microbial cells through hydroxyl and carboxyl groups. generated ROS with antimicrobial properties ,where *Candida albicans* exhibited higher sensitivity to ROS compared to *Aspergillus*, which possessed a more robust cell wall structure.



**Figure 6:** Antimicrobial Activity in *Candida albicans*

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**Figure 7:** Antimicrobial Activity in *Aspergillus* *Niger*

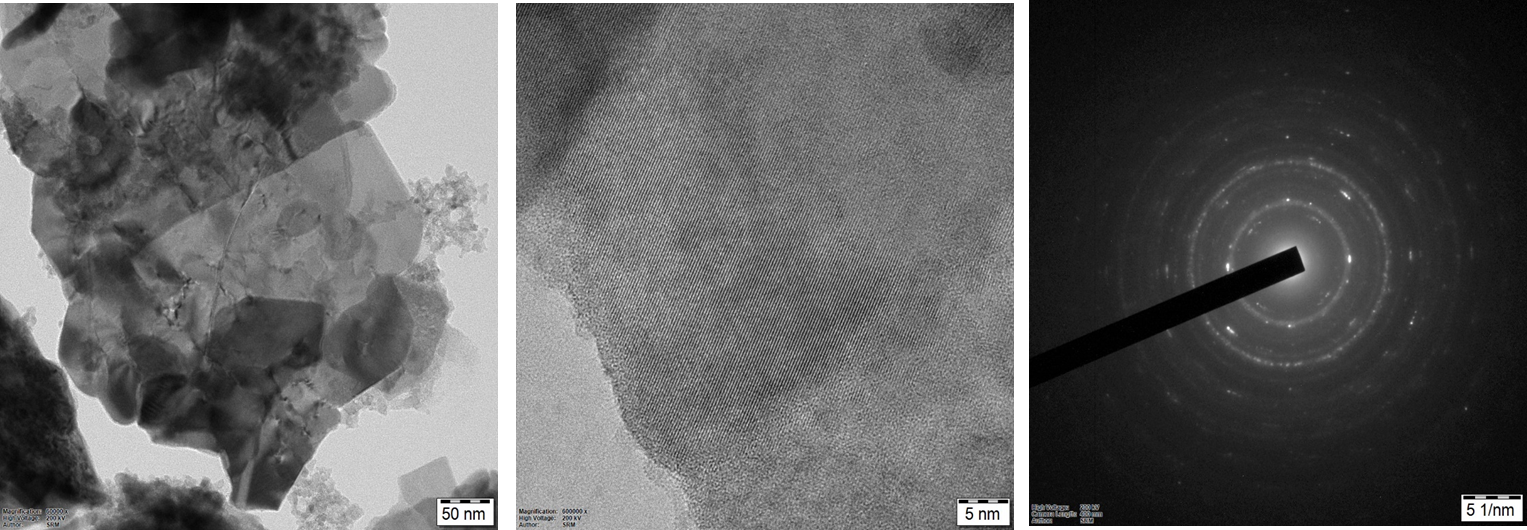
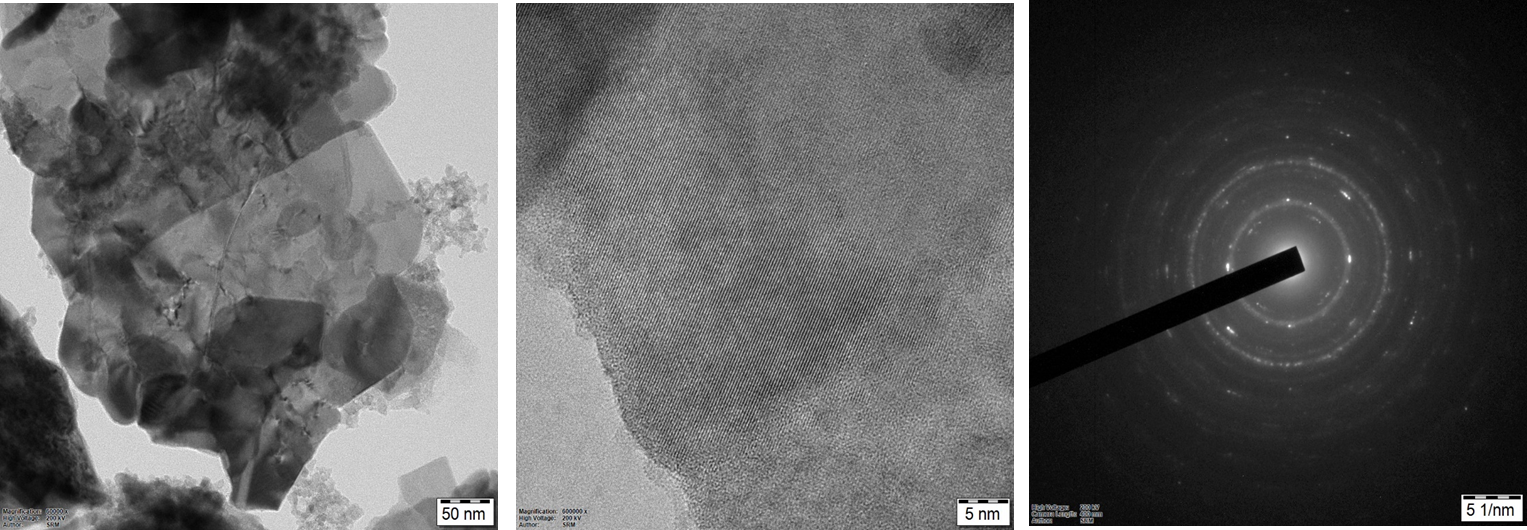


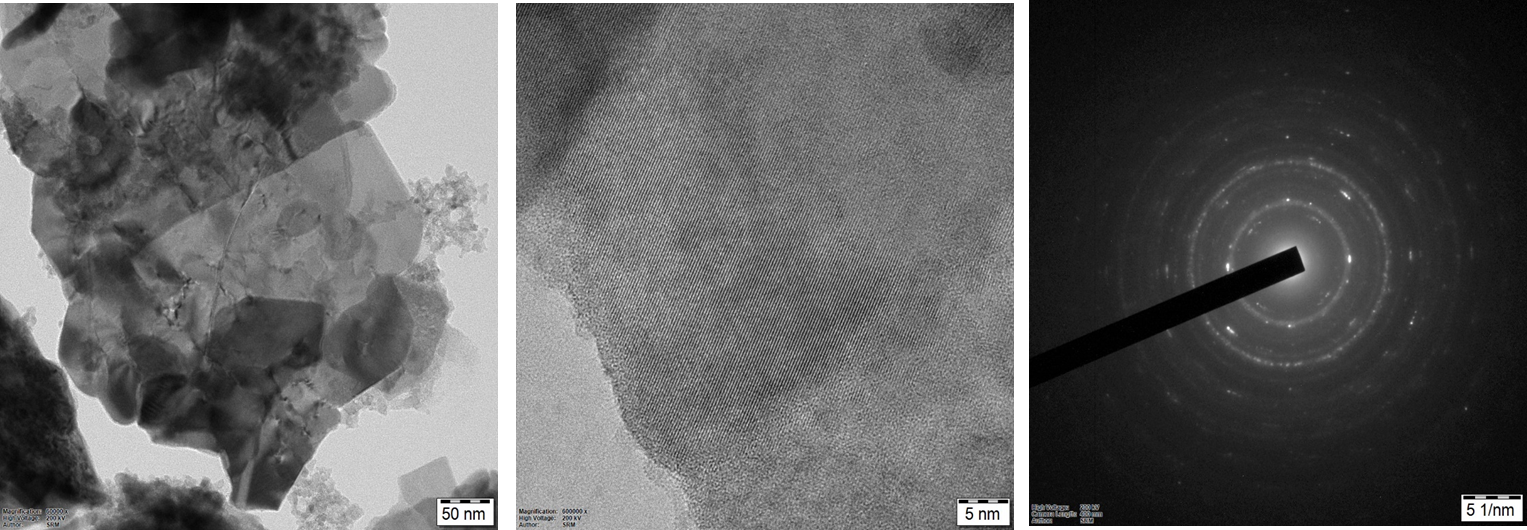
Fig: 8Transmission Electron Microscopy (TEM) Analysis

Transmission electron microscopy (TEM) analysis was performed to investigate the morphology of the Mo2C-NiS composites. The TEM images (Figure 1) reveal the presence of well-dispersed, rectangular nanoparticles with sharp edges were observed with an average diameter of approximately 50 nm. No significant aggregation of nanoparticles was observed[34,35][(Gavrilova et al., 2021)](https://paperpile.com/c/VybVEW/7sAlp)[(Xiong et al., 2016)](https://paperpile.com/c/VybVEW/Hr3ig).



**Figure 9:** High-Resolution Transmission Electron Microscopy (HR-TEM) Analysis

High-resolution TEM (HR-TEM) analysis (Figure 2) was performed to examine the crystal structure of the composite in more detail. The HR-TEM image reveals the presence of well-defined lattice fringes, indicating the crystalline nature of the Mo2C-NiS composites.[36,37][(Zhang et al., 2020)](https://paperpile.com/c/VybVEW/mxGgx)[(Xiong et al., 2016)](https://paperpile.com/c/VybVEW/Hr3ig).



**Figure 10:**Selected Area Electron Diffraction (SAED) Analysis:

Selected area electron diffraction (SAED) analysis (Figure 3) was performed to further investigate the crystallographic properties of the composite. The SAED pattern exhibits well-defined diffraction rings, indicating the polycrystalline nature of the composite(Rafi et al., 2024). Indexing of the diffraction rings revealed the presence of both Mo2C-NiS sulfide phases, consistent with the XRD analysis.[38,39,][(Huang et al., 2020)](https://paperpile.com/c/VybVEW/cBtyW)[(Singh et al., 2011)](https://paperpile.com/c/VybVEW/hRWiU)[(Mi et al., 2013)](https://paperpile.com/c/VybVEW/q1BQ0).

# Discussion

Our study is aimed to evaluate the antimicrobial mechanism of the nanocomposite synthesis with Molybdenum carbide and Nickel sulphide composite. The synthesis and distinct properties of the Mo2C-NiS composite were confirmed using UV-DRS ,XRD, FTIR, TEM, HR-TEM and SAED analysis . The antimicrobial mechanism was assessed by exposing the nanocomposite to Candida albicans and Aspergillus niger where it was observed to show significant antimicrobial mechanism which aligns with the known antimicrobial mechanism of the Mo2C-NiS composites from previous research articles.

The emergence of antifungal resistance is a growing concern, as it undermines the effectiveness of future treatments, similar to what has been observed with antibacterial drugs. There has been a rapid increase in the number of cases of drug-resistant pathogenic fungi over the past few decades [41][(Fisher et al., 2018)](https://paperpile.com/c/VybVEW/7FT4Q) .This rise in resistance to common antifungal agents has made it significantly more challenging to clinically treat fungal infections[(Elad et al., 1992; Fisher et al., 2018)](https://paperpile.com/c/VybVEW/7FT4Q+J95eG) . Of particular concern is the global spread of azole-resistant Aspergillus species and the increase in multidrug-resistant Candida species, as invasive infections caused by these species are associated with high mortality rates[(Sanguinetti & Lass-Flörl, 2015; van der Linden et al., 2015)](https://paperpile.com/c/VybVEW/CX1Me+4QTpU) . The widespread use of azoles, not only for human, animal, and crop health protection but also for antifouling coatings and wood preservatives, has accelerated the evolution of azole-resistant fungi [(Sabarathinam & Madhulaxmi, 2021)](https://paperpile.com/c/VybVEW/CbIP)[(Sushanthi et al., 2021)](https://paperpile.com/c/VybVEW/3A5K9)[(Harsha et al., 2022)](https://paperpile.com/c/VybVEW/zjHTt)[(Neha et al., 2021)](https://paperpile.com/c/VybVEW/qC5y)[(Maliael et al., 2021)](https://paperpile.com/c/VybVEW/uEQpT)[(Lakshmi, 2021)](https://paperpile.com/c/VybVEW/rncqG)[(Neha et al., 2021)](https://paperpile.com/c/VybVEW/qC5y)[(Dharman et al., 2021)](https://paperpile.com/c/VybVEW/CxNb3).Surveillance studies have shown an increase in the rates of resistant Candida species, with some institutional studies reporting even higher rates (Tuluwengjiang et al., 2024). For example, the prevalence of fluconazole-resistant Candida albicans infections in The Ninth People's Hospital of Chongqing in China increased dramatically from 36% to 64% in just two years.

# Conclusion

The current study exhibits that molybdenum carbide-nickel sulfide composites have promising antibacterial properties against Candida albicans and Aspergillus niger. The increased antibiotic efficacy demonstrated against Candida albicans highlights the significance of future investigation into the reasons affecting differential susceptibility. The proposed mechanism of action, which involves nanoparticle-cell interactions, cell membrane rupture, and reactive oxygen species formation, lays the groundwork for future mechanistic research. Characterization techniques validated the produced nanocomposites distinct features, setting the framework for enhancing their antibacterial potency. While this study provides valuable insights, additional in vivo investigations will be needed to determine clinical efficacy and safety. By addressing the necessary challenge of antibiotic resistance, these findings help to shape creative public health initiatives.

# Limitations

The study provides valuable insights into the potential of molybdenum carbide-nickel sulfide composites as antimicrobial agents. However, there are certain limitations that need to be addressed. Firstly, the focus on in vitro studies limits the direct application of the findings to in vivo conditions, and further research is required to bridge this gap. Additionally, a more comprehensive analysis of the underlying mechanisms is necessary for a deeper understanding of the antimicrobial properties of these composites. Furthermore, the study's use of a limited panel of bacterial strains emphasizes the need for a broader spectrum of microorganisms to ensure a more thorough and robust evaluation of the antimicrobial effectiveness.

# Future scope

Building on the findings of this study, future research can go deeper into the molecular understanding of antimicrobial action using advanced techniques such as time-kill kinetics and flow cytometry. In vivo studies can be performed to evaluate the efficacy and safety of these composites in animal infection models. Furthermore, investigating the potential of these composites as coverings for medical implants or components in drug delivery systems may broaden their applications. Optimizing the manufacturing process to produce regulated particle size and composition may improve antibacterial activity. Finally, investigation into the potential toxicity of these composites is critical for their safe clinical application.

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